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(54) Title: A DNA TRANSPORTER SYSTEM AND METHOD OF USE

(57) Abstract

A DNA transporter system capable of non-covalently binding to DNA and facilitating the insertion of the DNA into a cell. The DNA transporter system includes a binding complex which non-covalently binds the DNA. The binding complex includes a molecule that is capable of non-covalently binding to the DNA and being covalently linked to a surface ligand and to a nuclear ligand. The surface ligand is capable of binding to a cell surface receptor and the nuclear ligand is capable of recognizing and transporting the transporter system through the nuclear membrane. A plurality of these binding complexes are attached to the DNA to facilitate the transport of the DNA into the cell. Additionally, a third binding complex which includes a virus can also be non-covalently linked to the DNA. The virus facilitates the movement of the DNA through the cytoplasm and into the nucleus. Also described are a variety of structures which can be used as part of the transporter system as well as methods of using the transporter system to introduce DNA into cells.

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A DNA TRANSPORTER SYSTEM AND METHOD OF USE

The invention was partially supported by a grant from the United States government under HL-23741 awarded by the National Institute of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to transporter systems for inserting DNA into the cell nucleus. It more particularly relates to targeting DNA into the nucleus of specific cells, both *in vivo* and *in vitro*. Additionally, it relates to methods of using lytic peptides to release DNA into the cellular interior. Further, it relates to the methods of using a transporter system to insert DNA into cells.

BACKGROUND OF THE INVENTION

Generally it has been recognized that it is desirable to introduce genes into specific types of cells as therapy for human disease. A targeted gene delivery system for nonviral forms of DNA and RNA requires 4 components: (a) a DNA or RNA molecule with a known primary sequence that contains the genetic information of interest, (b) a moiety that recognizes and bind to a cell surface receptor or antigen, (c) a DNA binding moiety and (d) a lytic moiety that enables the transport of the entire complex from the cell surface directly into the cytoplasm. Current gene delivery systems are asialoorsomucoid or transferrin, covalently linked to either a polymer of the amino acid lysine or ethidium homodimer and then complexed with DNA. The polylysine or the ethidium moiety provides a positively charged template for noncovalent binding of the negatively charged nucleic acid expression vector. After cellular uptake of these complexes, detectable, but not quantitatively significant, amounts of the reporter gene have been found.

The principal limitation of this approach is the inability to prepare reproducible proteinaceous complexes in a consistent manner for gene delivery. The actual site of ligation is unknown. The covalent coupling of polylysine or the dye with proteins is nonspecific and gives a random mixture of conjugates. Because the conformation of positively charged surfaces is formed by chance, binding of DNA to the charged template is also variable. The high molecular weight and

variable stoichiometry of the components of the complex has made it difficult to prepare the complexes either consistently well or in sufficient quantity for *in vivo* delivery. The mixture of compounds precludes a molecular definition of the biologically active reagent and present formulations are inadequate for this reason.

The present invention is an improved delivery system using chemically defined template molecules. It provides an effective and efficient method to target gene delivery to specific cells. It further provides an effective and efficient method to release DNA into the cellular interior after the DNA has been internalized by the use of lytic peptides covalently linked to template molecules through an acid sensitive linker. It can also be used to target cells to the nucleus of the specific cells.

SUMMARY OF THE INVENTION

An object of the present invention is a transporter system capable of efficiently inserting DNA into the nucleus of a cell.

An additional object of the present invention is a compound useful as a DNA transporter system.

A further object of the present invention is a method of inserting genes into cells using a DNA transporter system.

Another object of the present invention is a method of releasing genes into the cellular interior after the DNA has been internalized by the use of lytic peptides covalently linked to template molecules through an acid sensitive linker.

Thus in accomplishing the foregoing objects, there is provided in accordance with one aspect of the present invention a DNA transporter system for inserting specific DNA into a cell comprising a plurality of a first DNA binding complex, said complex including a first binding molecule capable of non-covalently binding to DNA, said first binding molecule covalently linked to a surface ligand, said ligand capable of binding to a cell surface receptor; a plurality of a second DNA binding complex, said complex including a second binding molecule capable of non-covalently binding to DNA, said second binding molecule covalently linked to a nuclear ligand, said nuclear ligand capable of recognizing and transporting the transporter system through a nuclear membrane; wherein said plurality of first and second DNA binding complexes are capable of simultaneously, non-covalently binding to the specific DNA.

Specific embodiments the DNA transporter system of the present invention can further include a plurality of a third DNA binding complex, said complex including a third binding molecule capable of

non-covalently binding to DNA, said third binding molecule covalently linked to a virus or a lytic peptide; wherein said plurality of third DNA binding complexes are capable of simultaneously, non-covalently binding to the specific DNA.

In additional embodiments of the present invention, the binding molecules can be selected from the group consisting of spermine, spermine derivative, histones and polylysine.

In the preferred embodiment, a spermine derivative is used.

In specific embodiments of the present invention, the surface ligand is a molecule which binds to a receptor selected from the group consisting of a folate receptor, biotin receptor, lipoic acid receptor, low density lipoprotein receptor, asialoglycoprotein receptor, Fab' fragment of IgG, insulin-like growth factor type II/cation-independent mannose-6-phosphate receptor, calcitonin gene-related peptide receptor, insulin-like growth factor I receptor, nicotinic acetylcholine receptor, hepatocyte growth factor receptor, endothelin receptors or bile acid receptor. These receptors function in most, if not all, cell types, although their abundance varies considerably with cell type. Organ specificity can be achieved either by direct injection into the organ of interest or by intra-arterial administration with a venous outflow to remove any material not taken up by the tissue. In the preferred embodiment, the asialoglycoprotein receptor is used.

In the preferred embodiment, a spacer molecule is also used to link the binding complex to the surface ligand, the binding complex to the nuclear ligand and the binding complex to the virus. The spacer molecule is usually hydrophilic and ranges from 6 to 30 carbons. In the preferred embodiment, a compound from 6 to 16 carbons is used. A further embodiment is a spacer molecule that is polymer of $[(gly)_i(ser)_j]_k$ where i ranges from 1 to 6, j ranges from 1 to 6, and k ranges from 3 to 20.

The first, second and third DNA binding complexes can be the same common DNA binding complex where any combination of cell surface ligand, nuclear ligand, virus or lytic peptide can be linked to the same binding molecule.

Additional embodiments of the present invention include specific compounds which can be used as ligands in the transporter system.

Further embodiments of the present invention include methods of using the transporter system introducing DNA into cells; *in vivo* and *in vitro* targeting of the insertion of DNA into specific cells; prevention and treatment of disease; and modification of animals.

Other and further objects, features and advantages will be apparent from the following description of the presently preferred embodiments of the invention, which are given for the purposes of disclosure when taken in conjunction with the company drawings.

BRIEF DESCRIPTION OF THE DRAWING

Figures 1-3 show the schematic synthesis of the receptor ligands.

Figure 4 shows a schematic synthesis of the virus ligand.

Figures 5-13 show a schematic flow chart for the synthesis of the DNA transporter system.

Figure 14 is a schematic diagram of insertion of a triplex forming oligonucleotide or peptidyl nucleic acid by attachment of a ligand.

Figure 15A is a schematic representation of using a ligand to target to a triplex forming oligonucleotide to a duplex DNA. Figure 15A is a schematic representation using a ligand to target a triplex forming peptidyl oligonucleotide to a duplex DNA. Figure 15B is a specific ligand for targeting muscle. Figure 15C shows analogs useful in the compound of 15A. Figure 15D is an analog of 15A.

Figure 16 shows a specific targeting of a ligand for the SV40 sequences.

Figure 17 shows the targeting with a ligand of a sequence to the c-myc promoter region.

Figure 18 is a schematic of the peptide ligands and other ligands and shows the abbreviations used herein for the ligands.

Figure 19 shows the schematic synthesis of receptor ligands.

Figure 20 shows specific ligands for targeting to muscle.

Figure 21 shows the folyl-spermine derivative.

Figure 22 shows a specific ligand for targeting muscle.

Figure 23 is a schematic of the peptide ligands and other ligands and shows the abbreviations used herein for the ligands.

Figure 24 is a schematic representation of a synthetic route for bifunctional acid sensitive linkers.

Figure 25 is a schematic representation of a trimeric fusogenic peptide.

Figure 26 is a schematic representation of the synthetic route for a trimeric fusogenic peptide.

Figure 27 is a schematic representation of a synthetic route for a monomeric fusogenic peptide.

Figure 28 is a schematic representation of a synthetic route for a trimeric fusogenic peptide.

Figure 29 is a schematic representation of a synthetic route for a trimeric fusogenic peptide.

The drawings are not necessarily to scale. Certain features of the invention may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness.

DETAILED DESCRIPTION OF THE INVENTION

It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The term "DNA transporter" refers to a molecular complex which is capable of non-covalently binding to DNA and efficiently transporting the DNA through the cell membrane. Although not necessary, it is preferable that the transporter also transport the DNA through the nuclear membrane.

The term "spacer" as used herein refers to a chemical structure which links two molecules to each other. The spacer normally binds each molecule on a different part of the spacer molecule. In the present invention, the spacer is a hydrophilic molecule comprised of about 6 to 30 carbon atoms. In the preferred embodiment, it usually contains 6 to 16 carbons. In a second embodiment, the spacer is a hydrophilic peptide, a polymer of $[(gly)_i(ser)_j]_k$ where i ranges from 1 to 6, j ranges from 1 to 6, and k ranges from 3 to 20.

A receptor is a molecule to which a ligand binds specifically and with relatively high affinity. It is usually a protein or glycoprotein, but may also be a glycolipid, a lipopolysaccharide, a glycosaminoglycan or a glycocalyx. For this patent, the epitope to which an antibody or its fragment binds is construed as a receptor, since the antigen:antibody complex undergoes endocytosis.

The term "cell surface receptor" as used herein refers to a specific chemical grouping on the surface of a cell for which a ligand can attach. The receptor facilitates the internalization of the ligand and attached molecules. Cell surface receptors which have been found to be useful in the present invention include the folate receptor, the biotin receptor, the lipoic acid receptor, the low density lipoprotein receptor, the asialoglycoprotein receptor, IgG antigenic sites, insulin-like growth factor type II/cation-independent mannose-6-phosphate receptor, calcitonin gene-related peptide receptor, insulin-like growth factor I receptor, nicotinic acetylcholine receptor,

hepatocyte growth factor receptor, endothelin receptor, bile acid receptor.

The term "lytic peptide" refers to a chemical grouping which penetrates a membrane such that the structural organization and integrity of the membrane is lost. As the result of the presence of the lytic pepitde, the membrane undergoes lysis, fusion or both. Lytic peptides which have found to be useful in the present invention include those of the Othromyxoviridae, Alphaviridae, and Arenaviridae.

The term "nuclear receptor" refers to a chemical grouping on the nuclear membrane which will bind a specific ligand and help transport the ligand through the nuclear membrane. Nuclear receptors which have been found to be useful in the present invention include the receptors which bind the nuclear localization sequences.

As used herein, the term "ligand" refers to a chemical compound or structure which will bind to a receptor. In the present invention, useful cell surface ligands include folate (**A** Figure 1), lipoic acid (**G** Figure 3), biotin (**B** Figure 2), apolipoprotein E sequence (Pep2 Figure 18), **D** (Figure 18), Asp (bis-LacAHT) **E** (Figure 18), Fab' (Figure 18), Compound **P** L-tyrosyl-L-aspartoyl-*bis*-{N-[6-[[6-O-phosphoryl-α-D-mannopyranosyl]oxy]hexyl]-L-alaninamide] (Figure 19), Compound **J** 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid, For Peptides Pep12-19, X is the 3(2-pyridyldithio)propionyl moiety (Figure 20), Pep12. [Gln^0, Leu^{27}, ϵ-X-Lys^{67}]-insulin-like growth factor II, Pep13. [Gln^0, ϵ-X-Lys^3, Leu^{27}, Arg^{67}]-insulin-like growth factor II, Pep14. Y^0-ϵ-X-Lys^{24}-calcitonin gene-related peptide, Pep15. [$Asu^{2,7}$, Y^8, ϵ-X-K^{24}]-calcitonin gene-related peptide, Pep16. (Gln^0, Leu^{27}, ϵ-X-Lys^{54}, Arg^{55}, Arg^{67})-insulin-like growth factor II, Pep17. N-X-*des*-(1-3)-[Arg^{65}, Arg^{67}]-insulin-like growth factor I, Pep18. ϵ-X-K^0-thymopoietin, Pep19. ϵ-X-K^4-thymopoietin, $7\alpha,12\alpha$-dihydroxy-3β-(ω-aminoalkoxy)-5-β-cholan-24-oic acid, Pep20 hepatocyte growth factor, Pep21 endothelin-1, Pep22 N-succinyl-[glu^9,$ala^{11,15}$]-endothelin-1(8-21), Pep23 r-atrial natriuretic factor (99-126). In the preferred embodiment, **E** is used for liver and **P** is used for the muscle. One skilled in the art will readily recognize that the ligand chosen will depend on which receptor is being bound. Since different types of cells have different receptors, this provides a method of targeting DNA to specific types of cells, depending on which cell surface ligand is used. Thus, the preferred cell surface ligand may depend on the targeted cell type. In the present invention, useful lytic peptides are Pep24 influenza GLFEAIAGFIEDGWEGMIDGGGC, Pep25 SFV E1 KVYTGVYPFMWGGAYCFCD, and Pep26 Lassa gp2 GGYCLTRWMLIEAELKCFGNTAV. In the present invention, useful

nuclear ligands are shown on Figure 18 and include Pep3, Pep4, Pep5, Pep6, Pep7, Pep8, Pep9 and Pep10.

One embodiment of the present invention is a DNA transporter system for inserting specific DNA into a cell comprising a plurality of a first DNA binding complex, said complex including a first binding molecule capable of non-covalently binding to DNA, said first binding molecule covalently linked to a surface ligand, said surface ligand capable of binding to a cell surface receptor; a plurality of a second DNA binding complex, said complex including a second binding molecule capable of non-covalently binding to DNA, said second binding molecule covalently linked to a nuclear ligand, said nuclear ligand capable of recognizing and transporting a transporter system through a nuclear membrane; wherein said plurality of first and second DNA binding complexes are capable of simultaneously, non-covalently binding to a specific DNA.

Alternative embodiments of the present invention further included a plurality of a third DNA binding complex, said complex includes a third binding molecule capable of non-covalently binding to DNA, said third binding molecule covalently linked to a virus or lytic peptide; wherein said plurality of third DNA binding complexes are capable of simultaneously, non-covalently binding to a specific DNA.

The first binding molecule, the second binding molecule and third binding molecule can each be selected from the group consisting of spermine, spermine derivative, histones, cationic peptides and polylysine. Spermine derivative refers to analogues and derivatives of spermine and include compounds **IV**, **VII**, **XXI**, **XXXIII**, **XXXVI**, **LIV**, **LVI**, **LXXXII**, **LXXXIV** and **CX**. In the DNA transporter system, the first, second and third binding molecules can be different or can be the same. In the preferred embodiment, the first, second and third binding molecules are the same and are preferably a spermine derivative, most preferably **D**.

When the transporter enters the cell it is engulfed by an endosome. A virus or lytic peptide can be used to break down the endosomal membrane, freeing the transporter into the cytoplasm. The virus is usually selected from the group consisting of adenovirus, parainfluenza virus, herpes virus, retrovirus and hepatitis virus. In one preferred embodiment, the adenovirus of the structure F Figure 4 is used. The lytic peptide is selected from the peptides from proteins of the Othromyxoviridae, Alphaviridae, and Arenaviridae. In one preferred embodiment, the peptide of the structure Pep24.

The surface ligand, the nuclear ligand and the virus or lytic peptide can be attached directly to the first, second, and third binding molecules respectively; however, in the preferred embodiment, there is a spacer between the binding molecules and the ligands or virus. Some examples of binding molecules containing spacers which are useful in the present invention include compounds **XI, XII, XL, XLI, LX, LXI, LXXXVIII, XC, CXVIV, CXVI, XV, XVI, XVIII, XXI, XLV, LXVII, XLVII, L, LXV, LXX, XCIV, XCVI, XCIX, XXIV, XXV, LXXIII, LXXIV, CII, CV.**

Another embodiment of the DNA transport system for inserting specific DNA into a cell comprises a plurality of a common DNA binding complex. Each of said complexes includes a binding molecule capable of non-covalently binding to DNA, said binding molecule is attached to both a surface ligand capable of binding to a cell surface receptor and a nuclear receptor capable of recognizing and transporting the transporter system through a nuclear membrane. In preferred embodiments, this DNA transporter also includes at least one spacer which links the surface ligand and nuclear ligand to the binding molecule. Further embodiments of this DNA transporter include a virus, lytic peptide or Fab′ also attached to the common binding molecule. Again, either a virus or lytic peptide can be attached by the same or a different spacer.

The DNA transporter of the present invention can be used in a variety of methods for introducing DNA into the cell. One method comprises contacting the DNA with the elements of the transporter such that the transporter elements non-covalently bind to the DNA. This DNA transporter system is contacted with the cell for insertion. Another method involves the *in vitro* targeting of the insertion of DNA into a cell, In this procedure the DNA transporter system is contacted with the cell, however, the transporter has a cell surface ligand which is cell type specific. The invention can also be used for prevention and treatment of disease by introducing a therapeutic dose of DNA into the cells by contacting the DNA transporter system with the cells to be treated. This treatment, therapy and prevention can be used in both humans and in animals.

Another embodiment of the present invention is a method of modifying the genetic makeup of animals by introducing DNA into the cells of the animals. This can be used to modify production in domestic animals.

To make the DNA binding asialoglycoprotein receptor (ASGPR) Ligand, a derivative of the naturally occurring DNA binding ligand spermine is synthesized. This spermine derivative can be covalently

linked to a variety of cell specific ligands. The chemical structure of the spermine analog is IV. A spermine analog is coupled to a high affinity ligand for the ASGPR. The highest affinity for the ASGPR is achieved with two clusters of three galactose groups separated by an aspartic acid backbone. This structure Asp(bis-LacAHT) is E. The spermine derivative and the Asp(bis-LacAHT) are the two component molecules which are coupled to give the transporter.

This compound is used to define the structural features necessary for high efficiency *in vivo* delivery of the expression vector. One skilled in the art will recognize that this is only one of many compounds from which second or third generation products are derived. This DNA binding spermine derivative has a wide variety of applicability in gene delivery systems for a variety of cell types of interest (for example liver, muscle, endothelium and skin) as long as the specific cell surface receptors are present for targeting.

For cell specific target of expression vectors *in vivo* the system includes three components which are comprised of a DNA expression vector, a spermine derivative which binds the DNA to achieve a cell specific targeting, and pH sensitive liposomes or lytic peptides with a cell specific ligand to release the vector into the cytoplasm of the cell. The combination of these three components are taken up by the cell containing the receptors for both of the high affinity ligands. After co-internalization of the vector ligand complex and the lytic agent throughout the same coated pit on the plasma membrane of the cell, the decrease in pH that occurs immediately after endosome formation causes spontaneous lysis of the endosome. The vector is released into the cytoplasm for transfer to the nucleus where the targeted gene is expressed.

The pH sensitive liposomes have a second ligand specific for the same cell type as the DNA binding ligand. With the hepatocytes a ligand containing two copies of the apolipoprotein "E" sequence ("APO") Pep2 is used. *In vivo*, this ligand has high affinity for the low density lipoprotein receptor related protein on the hepatocyte plasma membrane.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1

Synthesis of Components of Receptor ligands

Examples of the specific components of the receptor ligands are shown in Figure 18 and Figures 1-4.

A. In Figures 1-3 the schematic synthesis of **A**, **B**, **G**, **P**, **J** and **M** are shown. The actual synthesis for **A**, **B**, **G**, **P**, **J** and **M** are very similar. As an example, for the preparation of **A**, dissolve 1 mmol of folic acid in 2 mL dry dimethylformamide (DMF), add 1.3 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carbodiimide and stir in the dark under N_2 overnight at 4°, then add 1.3 mmol N-hydroxysuccinimide with stirring continued for another 6 hr at 4°. Add bis(2-aminoethane)disulfide, 1 mmol in 0.5 mL dry dimethylformamide, dropwise to the reaction mixture and stir for an additional 4 hr. Add 15 mL water to precipitate the product. After centrifugation, the precipitate is washed and dissolved in oxygen-free 0.1 M NH_4OH. This solution is applied to an anion exchange resin and equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile. The γ-isomer is separated from unreacted starting materials and the α-isomer by chromatography in 0.1 M NH_4CO_3 containing 20% acetonitrile. The appropriate fractions are pooled and lyophilized to obtain the product.

The synthesis of the nicotinic acetylcholine receptor ligand, component J, 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid is the same as for folic acid, component A except the 1 mmol of folic acid 2 mL dry DMF is replaced with 1 mmol 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid in 2 mL dry DMF. The synthesis of the nicotinic acetylcholine receptor ligand, component J, 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid is the same as for folic acid, component A except the 1 mmol of folic acid 2 mL dry DMF is replaced with 1 mmol 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid in 2 mL dry DMF.

This compound can be further reacted to yield **A'**. Dissolve 2 mmol **A** in 10 mL oxygen-free 0.01 M NH_4CO_3 containing 2 mmol dithioerythritol, stir for 2 hr. The solution is applied to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The reduced folate derivative is separated from unreacted starting materials by chromatography in 0.1 M NH_4CO_3 containing 20% acetonitrile. The appropriate fractions are pooled, lyophilized, and then dissolved in 10 mL dry dimethylformamide for dropwise addition to a vigorously stirred solution of 2,2'-dipyridinedisulfide, 4 mmol dissolved in 10 mL ethanol containing 0.4 mL glacial acetic acid. After overnight at room temperature protected from light, the solvent is removed *in vacuo*. Add degassed 0.1 M NH_4CO_3 to effect solution and then chromatograph as before to obtain the desired product. Both the

original A, B and G and the further reacted A', B' and G' have been used. The synthesis of the nicotinic acetylcholine receptor ligand, component J, 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid is the same as for folic acid, component A except the 1 mmol of folic acid 2 mL dry DMF is replaced with 1 mmol 3-{N-[3,4,5-*tris*-(2-triethylammoniumethoxy)benzoic acid in 2 mL dry DMF.

One skilled in the art will recognize that other vitamins and analogs of these vitamins can be used. Since the different vitamins and analogs will have different affinities, uptake and selectivity for the membrane receptors, the specific vitamin or analog is chosen to maximize the specificity and uptake.

B. The peptides including Pep1 through Pep11 and Pep21 through 26 can be synthesized by a variety of methods. In the present invention solid phase synthesis on a support is preferred, except for peptides 12, 13, 16, 17 and 20 which are recombinant proteins produced by expression vectors in bacteria, yeast, baclovirus or mammalian systems.

Peptides Pep1 through Pep6 and Pep12 through 23 are examples of peptides or peptide analogs. These peptides target and bind to membrane receptors. One skilled in the art recognizes that other peptides or analogs to other membrane receptors can be used, and that the order of the amino acid sequence can be reversed, inverted and/or repeated, while still maintaining the transporter characteristics. The selection of a specific peptide will depend on the tissue and membrane receptor which is targeted. By selecting specific peptides, one skilled in the art recognizes the binding efficiency, uptake and specificity can be regulated. This can be used for tissue specificity.

Peptides Pep24 through Pep26 are examples of peptides or peptide analogs. These peptides lyse membranes. One skilled in the art recognizes that other peptides or analogs to other lytic peptides can be used, and that the order of the amino acid sequence can be reversed, inverted and/or repeated, and still maintain the lytic characteristics. The selection of a specific peptide will depend on the tissue and membrane receptor which is be targeted.

I. SPERMINE TEMPLATE

a. monomeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

$NH_2-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NH_2$
$\quad\quad\quad\quad\quad\quad\quad\quad | $
$\quad\quad\quad\quad\quad\quad\quad\quad OCH_2CONH-CH_2CH_2-NH-R$ (9)

where R = Pep24-SS-CH_2CH_2NH-α-CO-aconityl-γ-CO-

$H_2N-(CH_2)_3-NH-CH_2-CH(OH)-(CH_2)_2-NH-(CH_2)_3-NH_2$ + $C_{15}H_{11}OCO-Cl$ (IV)

↓

FMOC-NH-$(CH_2)_3$-N(FMOC)-CH_2-CH(OH)-$(CH_2)_2$-N(FMOC)-$(CH_2)_3$-NH-FMOC (1)

+ $BrCH_2COOCH_3$, $NaOCH_3$

↓

FMOC-NH-$(CH_2)_3$-N(FMOC)-CH_2-CH(OCH_2COOCH_3)-$(CH_2)_2$-N(FMOC)-$(CH_2)_3$-NH-FMOC (2)

↓ + OH^-

FMOC-NH-$(CH_2)_3$-N(FMOC)-CH_2-CH(OCH_2COOH)-$(CH_2)_2$-N(FMOC)-$(CH_2)_3$-NH-FMOC (3)

↓ + $H_2NCH_2CH_2NH_2$

FMOC-NH-$(CH_2)_3$-N(FMOC)-CH_2-CH($OCH_2CONHCH_2CH_2NH_2$)-$(CH_2)_2$-N(FMOC)-$(CH_2)_3$-NH-FMOC (4)

↓ + 6

FMOC-NH-$(CH_2)_3$-N(FMOC)-CH_2-CH-$(CH_2)_2$-N(FMOC)-$(CH_2)_3$-NH-FMOC (7)

with side chain $OCH_2CONHCH_2CH_2NH$-γ-CO-*cis*-$C_4H_4O_2$-CONH$(CH_2)_2$SS-C_5H_4N

↓ + piperidine

$H_2N-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NH_2$ (8)

with side chain $OCH_2CONHCH_2CH_2NH$-γ-CO-*cis*-$C_4H_4O_2$-CONH$(CH_2)_2$SS-C_5H_4N

↓ + influenza fusion peptide

GLFEAIADFIENGWEGMIDGGGC-SH (Pep24)

$$NH_2-(CH_2)_3-NH-CH_2-\underset{\displaystyle OCH_2CONH-CH_2CH_2-NH-R}{\underset{|}{CH}}-(CH_2)_2-NH-(CH_2)_3-NH_2 \qquad (9)$$

where R = GLFEAIADFIENGWEGMIDGGGC-SS-CH_2CH_2NH-α-CO-aconityl-γ-CO-

Detailed Preparative Procedures.

Combine 2 mmol (S)-hydroxyspermine (**IV**), 4 mmol 4-pyrrolidinopyridine and 4.1 mmol 9-fluorenylmethyl chloroformate in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product (1) by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

Add 4 mL of dry benzene to 1 mmol K_2CO_3 and 2 mmol 18-crown-6 and stir for 20 min. Add 2 mmol 1 in 4 mL of dry benzene, followed by 2 mmol methyl bromoacetate in 2 mL benzene. After 4 hr, add 25 mL of water and extract with 3 portions of 25 mL benzene. Remove the solvent *in vacuo* and dissolve the residue in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 2 mmol of HCl, 5 mL of water and 25 mL of benzene is added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*, redissolved in a minimum volume of ethyl acetate, diluted with enough petroleum ether to create slight turbidity and cooled at 4° to promote crystallization of 3.

Dissolve 1 mmol of 3 in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide and stir 2 hr, then add 1.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature. This solution is added dropwise to 10 mmol of 1,2-diaminoethane in 0.5 mL dry dimethylformamide, and stirring continued for an additional 4 hr, when the reaction is complete as monitored by thin layer chromatography. The solution is applied to an cation exchange resin equilibrated in degassed water. The product is separated from unreacted starting materials by a gradient from 0.0005 to 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain the product (4).

The Synthetic route for the bifunctional acid sensitive linker (6) is shown in Figure 24.

Combine 10 mmol *cis*-aconityl anhydride 10 mmol with 2-(2'-aminoethyldithio)-pyridine in 20 mL dry DMF under N_2 protected from light at room temperature for 18 hr. Remove the solvent *in vacuo*,

dissolve the residue in the minimum amout of 3M NH_4CO_3, dilute 10 fold and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 and elute with a gradient to 1.0M NH_4CO_3. The appropriate fractions are pooled, lyophilized and the residue (5) dissolved in 2-propanol and crystallized at 4° after the solution is made turbid by the addition of diethyl ether. The product (5) is collected by filtration.

Dissolve 1 mmol of 5 in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 24 hr at 4°. Remove the solvent *in vacuo*, dissolve the residue in the minimum amout of 2-propanol and crystallize at 4° after the solution is made turbid by the addition of diethyl ether. The product (6) is collected by filtration.

Dissolve 1 mmol of 6 in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 6 hr at 4°. One mmol of 4 in 0.5 mL dry dimethylformamide, is added dropwise to the preceding reaction mixture and stirring continued for an additional 4 hr. Remove the solvent *in vacuo* and dissolve the residue in 10 mL 50% piperidine in DMF (v/v). Again remove the solvents *in vacuo*, solubilize the residue (7) in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 0.25 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (8).

To a stirred solution of 0.5 mmol of lytic peptide (Pep24) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of 8 in PBS dropwise. After 18 hr, chromatograph over a molecular sieve to separate the product (9) from unreacted starting materials.

b. trimeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

$$\begin{array}{l} NH_2-(CH_2)_3-NH-CH_2-\underset{\displaystyle |}{CH}-(CH_2)_2-NH-(CH_2)_3-NH_2 \\ OCH_2CONH-CH_2CH_2-NH-R \end{array} \qquad (16)$$

where R = $-\alpha$-CO-aconityl-γ-COHNC-$(OCH_2CH_2NHCO(CH_2)_5NH-R')_3$
and R' = Pep24-SS-CH_2CH_2CO-

The Series II synthetic route (New compounds 10 - 16) is shown in Figure 25.

Detailed procedures.

Add 4 mL of dry benzene to 1 mmol K_2CO_3 and 2 mmol 18-crown-6 and stir for 20 min. Add 2 mmol N-t-BOC-*tris*-(hydroxymethyl)methane in 4 mL of dry benzene, followed by 20 mmol 1-bromo-2-(N-5-FMOC-aminohexanoyl)-aminoethane in 2 mL benzene. After 4 hr, add 25 mL of water and extract with 3 portions of 25 mL benzene. Remove the solvent *in vacuo* and dissolve the residue (**10**) in 10 mL 50% piperidine in DMF (v/v). After 6 hr, remove the solvents *in vacuo*, solubilize the residue in 20 mL ethyl acetate, wash with water until neutral and dry over molecular sieve before solid phase extraction and chromatography on octadecyl-silica, using a gradient of acetonitrile to 100%. Pool the appropriate fractions to obtain **11**.

Combine 2 mmol **11** in 20 mL acetonitrile with 7 mmol of succinimidyl 3(2-pyridyldithio)-propionate in ethanol. After 60 min, dilute with sufficient water to create a slight turbidity and apply to octadecyl-silica, again using a gradient of acetonitrile from 0 to 100%. Pool the appropriate fractions to obtain **12**.

Dissolve 1 mmol **12** in 20 mL 3N HCl at 4° and allow to stand for 60 min before the solution is taken to dryness *in vacuo*. The residue is resuspended in water and solublized with the minimum amount of acetonitrile and chromatographed on octadecyl-silica, again using a gradient of acetonitrile from 0 to 100%. Pool the appropriate fractions to obtain **13**.

Dissolve 1 mmol of **13** in 2 mL dry DMF, add 3 mmol *cis*-aconityl anhydride and stir under N_2 overnight at 4°. Remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 0.25 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**14**).

Dissolve 1 mmol of **14** in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 6 hr at 4°. One mmol of **4** in 0.5 mL dry dimethylformamide, is added dropwise to the preceding reaction mixture and stirring continued for an additional 4 hr. Remove the solvent *in vacuo* and dissolve the residue in 10 mL 50% piperidine in DMF (v/v). Again remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 0.25 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**15**).

To a stirred solution of 0.5 mmol of lytic peptide (**Pep24**) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of **15** in PBS dropwise. After 18 hr, chromatograph over a molecular sieve to separate the product (**16**) from unreacted starting materials.

c. trimeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

$$NH_2\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{\displaystyle |\atop \displaystyle OCH_2CONH\text{-}CH_2CH_2\text{-}NH\text{-}R''}{CH}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$$

where R" =

$$\text{-}\alpha\text{-CO-aconityl-}\gamma\text{-COHN-glu-}\overset{\epsilon\text{-}R'}{\overset{|}{\text{lys}}}\text{-gly-}\overset{\epsilon\text{-}R'}{\overset{|}{\text{lys}}}\text{-gly-pro-gly-gly-}\overset{\epsilon\text{-}R'}{\overset{|}{\text{lys}}}\text{-}\alpha,\gamma\text{-diaminobutyric-}CONH_2 \qquad \textbf{(20)}$$

with the γ-carboxyl of glu[1] in amide linkage with the γ-amino of α,γ-diaminobutyric acid[10]

and R' = Pep24-SS-CH_2CH_2CO-

The **Series III synthetic route (New compounds 17 - 20)** is shown in Figure 26.

Detailed experimental procedures.

Solid phase peptide synthesis with conventional reagents and procedures gives **17**. It is obvious to one skilled in the art that homologs of 2,4-diaminobutyric acid, such as orinithine and lysine, could be substituted for this residue and that other amino acids, such as serine, alanine, and aspartic acid, could be substituted for gly.

Dissolve 1 mmol of **17** in 2 mL dry DMF, add 3 mmol *cis*-aconityl anhydride and stir under N_2 overnight at 4°. Remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 0.25 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**18**).

Dissolve 1 mmol of **18** in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 6 hr at 4°. One mmol of **4** in 0.5 mL dry dimethylformamide, is added dropwise to the preceding reaction mixture and stirring continued for an additional 4 hr. Remove the solvent *in vacuo* and dissolve the residue in 10 mL 50% piperidine in DMF (v/v).

Again remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 0.25 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**19**).

To a stirred solution of 0.5 mmol of lytic peptide (**Pep24**) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of **19** in PBS dropwise. After 18 hr, chromatograph over a molecular sieve to separate the product (**20**) from unreacted starting materials.

a. monomeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

```
tyr-lys-ala-(lys)40trp-lys-CONH2
     |
     NHCO-CH2-CH2-CONH-CH2CH2-NH-R                    (24)
```

where R = Pep24-SS-CH_2CH_2NH-α-CO-aconityl-γ-CO-

The **Series IV synthetic route (New compounds 21 - 24)** is shown in Figure 27.

Detailed experimental procedures.

Solid phase peptide synthesis with conventional reagents and procedures gives **21**.

Dissolve 1 mmol of **21** in 2 mL dry DMF, add 3 **6** and stir under N_2 overnight at 4°. Remove the solvents *in vacuo*, solubilize the residue in acetonitrile and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 1.0 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**22**).

Dissolve 1 mmol of **22** in 10 mL 50% piperidine in DMF (v/v). Remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 and elute with a gradient to 1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**23**).

To a stirred solution of 0.5 mmol of lytic peptide (**Pep24**) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of **23** in PBS dropwise. After 18 hr, chromatograph over a molecular sizing column to separate the product (**24**) from unreacted starting materials.

b. trimeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

```
tyr-lys-ala-(lys)40trp-lys-CONH2
     |
     NHCO-CH2-CH2-CONH-CH2CH2-NH-R                    (27)
```

where R = $-\alpha$-CO-aconityl-γ-COHNC-$(OCH_2CH_2NHCO(CH_2)_5NH\text{-}R')_3$
and R' = Pep24-SS-CH_2CH_2CO-

The **Series V synthetic route (New compounds 25 -27)** is shown in Figure 28.

Detailed experimental procedures.

Dissolve 1 mmol of **14** in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 6 hr at 4°. One mmol of **21** in 0.5 mL dry dimethylformamide, is added dropwise to the preceding reaction mixture and stirring continued under N_2 overnight at 4°. Remove the solvents *in vacuo*, solubilize the residue in acetonitrile and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 1.0 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**25**).

Dissolve 1 mmol of **25** in 10 mL 50% piperidine in DMF (v/v). Remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 and elute with a gradient to 1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**26**).

To a stirred solution of 0.5 mmol of lytic peptide (**Pep24**) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of **23** in PBS dropwise. After 18 hr, chromatograph over a molecular sizing column to separate the product (**27**) from unreacted starting materials.

c. trimeric fusogenic peptide covalently linked to a polycation through an acid sensitive, reducible spacer.

```
tyr-lys-ala-(lys)40trp-lys-CONH2
     |
     NHCO-CH2-CH2-CONH-CH2CH2-NH-R"                   (30)
```

where R" =

```
                   ε-R'    ε-R'                 ε-R'
                    |       |                    |
-α-CO-aconityl-γ-COHN-glu-lys-gly-lys-gly-pro-gly-gly-lys-α,γ-diaminobutyric-CONH2
```

with the γ-carboxyl of glu[1] in amide linkage with the γ-amino of α,γ-diaminobutyric acid[10]
and R' = Pep24-SS-CH_2CH_2CO-

The Series VI synthetic route (New compounds 28 -30)is shown in Figure 29.

Detailed experimental procedures.

Dissolve 1 mmol of **18** in 2 mL dry DMF, add 1.1 mmol N-hydroxysuccinimide and 1.1 mmol dicyclohexylcarbodiimide and continue stirring for another 6 hr at 4°. One mmol of **21** in 0.5 mL dry dimethylformamide, is added dropwise to the preceding reaction mixture and stirring continued under N_2 overnight at 4°. Remove the solvents *in vacuo*, solubilize the residue in acetonitrile and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 containing 20% acetonitrile and eluted with a gradient of 0 to 1.0 M acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**28**).

Dissolve 1 mmol of **28** in 10 mL 50% piperidine in DMF (v/v). Remove the solvents *in vacuo*, solubilize the residue in 0.1 M NH_4OH, and apply to an anion exchange resin equilibrated in degassed 0.05 M NH_4CO_3 and elute with a gradient to 1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product (**29**).

To a stirred solution of 0.5 mmol of lytic peptide (**Pep24**) in 5 mL PBS, pH 7.4, at 4°, add 0.1 mmol of **29** in PBS dropwise. After 18 hr, chromatograph over a molecular sizing column to separate the product (**30**) from unreacted starting materials.

Peptides such as Pep7 through Pep10 are nuclear localization sequences which are used to target the inserted DNA to the nucleus. One skilled in the art recognizes that the peptides shown in Figure 18 are only examples of this class of peptides and that there are a wide variety of other nuclear localization sequence peptides which can be used.

H_2N-Tyr-ε-N-lys-Pep11-CONH, and γ-N-[N-12-methoxy-6-chlorodiridinyl-HN-tyr-ε-N-lys-Pep11-CO]-2-N-(2-methoxy-6-chloroacridinyl) diaminobutanoyl-$COHN_2$ were prepared by standard solid phase peptide synthesis. One skilled in the art recognizes that any amino acid polymer such as H_2N-$(lys)_n$-COOH, H_2N-$(arg\text{-}ala)_n$-COOH, histones and other DNA binding cationic polypeptides and proteins which form an

α-helix, can be substituted for the lys-ala template. The -NH-(lys-ala)$_n$-CO unit can be extended. The useful range is from 2 to greater than 100 depending on the sequence of the inserted DNA, the target, uptake and specificity. The sequence position of the ε-N-substituted-lys residue can be either amino-terminal or carboxyl-terminal. The substitution can be any amino reactive DNA binding dye as well as the acridine moiety. Examples of DNA binding dyes include thiaxanthenones, lucanthone, hycanthone, phenanthrenemethanol, metallointercalation reagents, tilorone, napthiophene, phenanthridiniums, dimidium, ethidium, propidium, quinacrine.

In further embodiments, the spacers can be attached to the α-amino group of the N-terminal amino acid, and/or the carboxyl group of the C-terminal amino acid, rather than the ε-amino group of lysine, to reduce immunological response to the ligand.

C. In addition to the above components, it has also been found that fusion competent virus can be used to target the inserted DNA. Figure 4 shows the schematic procedure for preparing a fusion competent virus for use in the present invention. A variety of fusion competent virus can be used. As an example, adenovirus can be prepared in two separate ways. To a stirred solution of 10 mg of fusion competent adenovirus in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 20 mM succinimidyl 3(2-pyridyldithio)propionate in ethanol dropwise. After 60 min, dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr. Alternatively, to a stirred solution of 10 mg of fusion competent adenovirus in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 20 mM 2-iminothiolane HCl in ethanol dropwise. After 60 min, dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.

D. Recombinant Peptides 12, 13, and 16, 10 mg, prepared and purified by published methods (Bayne et al., J.Biol.Chem. 264:11004-11008, 1988), are dissolved in 10 mL 50 mM NH_4OH, pH 8.5. Aliquots are removed at 2 hr intervals to determine the extent of cyclization of N-terminal glutamine to pyroglutamate. When this reaction is complete, the solution is lypholyzed and then dissolved in 5 mL PBS, pH 7.4. The Peptides 12, 13, 16, and 17 are further reacted with either succinimyl 3(2-pyridyldithio) propionate or 2-iminothiolane as described for adenovirus (Example 1C).

E. Further, it has been found that either monoclonal or polyclonal IgG can be used to target the inserted DNA. Generally, the IgG is cleaved with immobilized pepsin to yield (Fab'-S-)$_2$ which is selectively reduced to Fab'-SH. Specifically, this includes: adding dropwise 0.5 mL 0.1 mM dithiothreitol to a stirred 5 mL solution of 1

nmol IgG F(ab')$_2$ at 4°, which was prepared by standard methods with immobilized pepsin. After 60 min, dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.

F. Synthesis of component D. Standard continuous-flow solid phase synthetic methodologies are used to prepare H_2N-his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu-gly-$CONH_2$, which is released as a protected peptide, containing N^{im}-Fmoc-his, N^9-4-methoxy-2,3,6-trimethylphenylsulfonyl-arg, and glu-γ-Fmoc ester. Coupling of this protected peptide using DCCI with the appropriate protected peptide on the solid support, gives

t-BOC-NH-CH-CONH-$(CH_2)_5$COHN-Pep1-COO-resin
|
CH_2-CONH$(CH_2)_5$COHN-Pep1-$CONH_2$.

Deprotection of the asp-amino group, reaction with succinimidyl 3(2-pyridyldithio)propionate, deblocking and cleavage from the resin gives D.

G. Synthesis of the asialoglycoprotein receptor ligand, component E. Dissolve 1 mmol N^{α},N^{β}-bis{hexanamido[*tris*-(β-lactosylhydroxymethyl)methane]}aspartyl diamide (Meth. Enzymol. 138:424-429, 1987), in 20 mL PBS, pH 7.4, and combine with 1 mmol succinimydyl 3(2-pyridyldithio) propionate, in 2 mL phosphate-buffered saline, pH 7.4. Dilute the reaction mixture 20-fold with water, apply to a cation exchange column to separate the desired product from unreacted starting material and other products, using a linear gradient formed from equal volumes of water and 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain the product, E.

H. Preparation of Compound I, L-tyrosyl-L-aspartoyl-*bis*-{N-[6-[[6-O-phosphoryl-α-D-mannopyranosyl]oxy]hexyl]-L-alaninamide] is prepared as described (Carb. Res. 198:235-246 (1990), except that N-*t*-BOC-L-tyrosine is used *in lieu* of N-acetyl-L-tyrosine. Compound I is further reacted as described for Compound E. (Example 1G).

Example 2

Synthesis of Tetracationic DNA Binding Templates

The overall schematic flow chart for the synthesis of these compounds is shown in Figure 5. The chemical pathway of synthesis is shown below. The Roman numerals are used to identify the specific compounds.

Dissolve 2 mmol of free base 1,4-diaminobutan-2-ol (II) (Meth. Enzymol. 94:431-433, 1983), in 5 mL of ethanol, add 4.1 mmol of acrylonitrile and allow to stand overnight at room temperature. Cool in an ice bath and saturate the solution with anhydrous NH_3 at 0°. Add about 5 mL of sponge nickel hydrogenation catalyst and shake under H_2 on a Paar low-pressure hydrogenator until the theoretical amount of H_2 is consumed. Remove the catalyst by filtration and wash the catalyst with ethanol. Combine filtrate and washings, then remove the ethanol *in vacuo*. Chromatograph on a cation exchange column to separate the desired product (**R and S IV**) from unreacted starting material and other products, using a linear gradient formed from equal volumes of water and 2.0 M HCl. Resolve the enantiomers of **IV** on a chiral column such as (R)-N-3,5-dinitrobenzoylleucine-silica (Baker) by a gradient of 2-propanol, from 0 to 20% in hexane.

$$Br\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{C}H}\text{-}CH_2CH_2\text{-}Br \qquad (I) \quad + \quad NH_4OH$$

$$\downarrow$$

$$H_2N\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{C}H}\text{-}CH_2CH_2\text{-}NH_2 \qquad (II)$$

$$\downarrow \quad CH_2{=}CH\text{-}CN$$

$$CN\text{-}(CH_2)_2\text{-}NH\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{C}H}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_2\text{-}CN \qquad (III)$$

$$\downarrow$$

$$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{C}H}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2 \qquad (IV)$$

Next combine 2 mmol (S)-hydroxyspermine (IV), 8 mmol 4-pyrrolidinopyridine and 8.2 mmol benzyloxycarbonyl anhydride (Z_2O)in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product (**V**) by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

$$IV \quad + \quad Z_2\text{-}O$$

$$\downarrow$$

$$\text{Z-NH-}(CH_2)_3\text{-}\underset{|}{\overset{Z}{N}}\text{-}CH_2\text{-}\underset{OH}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{N}\text{-}(CH_2)_3\text{-NH-Z} \quad \textbf{(V)}$$

$$+\ BrCH_2COOCH_3,\ NaOCH_3$$

$$\downarrow$$

Add 4 mL of dry benzene to 1 mmol K_2CO_3 and 2 mmol 18-crown-6 and stir for 20 min. Add 2 mmol **V** in 4 mL of dry benzene, followed by 2 mmol methyl bromoacetate in 2 mL benzene. After 4 hr, add 25 mL of water and extract with 3 portions of 25 mL benzene. Remove the solvent *in vacuo* and dissolve the residue (**IV**) in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 2 mmol of HCl, 5 mL of water and 25 mL of benzene are added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*, redissolved in a minimum volume of ethyl acetate, dried over 10% w/v anhydrous Na_2SO_4 overnight. The organic phase is decanted and diluted with a sufficient amount of petroleum ether to create slight turbidity and cooled at 4° to promote crystallization of **VII** (1,4,9,12-tetrabenzyloxycarbonyl-1,12-diamino-6-carboxymethoxy-4,9-diazadodecane).

$$\text{Z-NH-}(CH_2)_3\text{-}\overset{Z}{N}\text{-}CH_2\text{-}\underset{OCH_2COOCH_3}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{N}\text{-}(CH_2)_3\text{-NH-Z} \quad \textbf{(VI)}$$

$$\downarrow \quad +\ OH^-$$

$$\text{Z-NH-}(CH_2)_3\text{-}\overset{Z}{N}\text{-}CH_2\text{-}\underset{OCH_2COOH}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{N}\text{-}(CH_2)_3\text{-NH-Z} \quad \textbf{(VII)}$$

Dissolve 1 mmol of **VII** in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)] carbodiimide, stir 2 hr, then add 1.1 mmol N-hydroxysuccinimide and stir for an additional 6 hr at room temperature. This solution is added dropwise to 1 mmol of **A** in 0.5 mL dry dimethylformamide. Stirring is continued in the dark under N_2 for an additional 4 hr. When the reaction is complete, as monitored by thin layer chromatography, 15 mL oxygen-free water is added to precipitate the product. The product is collected by centrifugation, washed and dissolved in oxygen-free 0.1 M NH_4OH. The solution is applied to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The γ-isomer is separated from unreacted

starting materials and the α-isomer by a gradient from 20% to 50% acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product. Alternative compounds in this series, for example **VIIIb** and **VIIIc**, are made by substituting the appropriate starting material containing biotin, lipoic acid or other substituent.

VII + $H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$

Where R is **A**, **B**, **G**, **P**, **J**, **Pep 12 through Pep 26** or H.

↓

$$\text{Z-NH-}(CH_2)_3\text{-}\underset{}{\overset{\displaystyle Z\\|}{N}}\text{-}CH_2\text{-}\underset{\displaystyle |\\ OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R}{CH}\text{-}(CH_2)_2\text{-}\overset{\displaystyle Z\\|}{N}\text{-}(CH_2)_3\text{-NH-Z}$$

Where R is **A** (**VIIIa**), **B** (**VIIIb**), **G** (**VIIIc**) or H (**IX**).

Dissolve 1 mmol **VIIIa** or **VIIIb** or **VIIIc** or **IX** in 20 mL glacial acetic acid containing 30% HBr and stir overnight at room temperature in the dark under N_2. Add 30 mL diethyl ether to precipitate the product. Wash the product until the odor of acetic acid is gone. Dissolve the solid in oxygen-free 0.1 M NH_4OH and apply to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product **Xa** or **Xb** or **Xc** or **XI** is separated from unreacted starting materials by a gradient of 0 to 90% acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product.

$$NH_2\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{\displaystyle |\\ OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R}{CH}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$$

Where R is **A** (**Xa**), **B** (**Xb** , **G** (**Xc**) or H (**XI**).

Dissolve 2 mmol **XI** purified by chromatography as was done for **IV**, in 10 mL oxygen-free 0.01 M NH_4CO_3 containing 2 mmol dithiothreitol and stir for 2 hr. Bring the solution to pH 5 with 1N HCl and apply the solution to a cation exchange resin equilibrated in degassed water. The product **XII** is isolated by a gradient from 0.0005 M to 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain the product. This is followed by reactions described above to yield **XIIId**, **XIIIe** and **XIIIf**.

XI + dithiothreitol

↓

$NH_2-(CH_2)_3-NH-CH_2-CH(OCH_2CONH-CH_2CH_2-SH)-(CH_2)_2-NH-(CH_2)_3-NH_2$ (**XII**)

↓ $C_5H_4N-S-S-CH_2CH_2-CONH-R$; Where R is **D**, **E** or **F**.

$NH_2-(CH_2)_3-NH-CH_2-CH(OCH_2CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-R)-(CH_2)_2-NH-(CH_2)_3-NH_2$

Where R is **D** (**XIIId**), **E** (**XIIIe**) or **F** (**XIIIf**).

Dissolve 1 mmol **XII** in 2 mL phosphate-buffered saline, pH 7.4, and combine with 1 mmol of further reacted (**see above**) **A**, **B** or **G** in 2 mL phosphate-buffered saline, pH 7.4. Dilute the reaction mixture 20-fold with water, apply to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product **XIa**, **XIb** or **XIc** is separated from unreacted starting materials by chromatography in 0.1 M NH_4CO_3 containing 20% acetonitrile. The appropriate fractions are pooled and lyophilized to obtain the product.

XII + $C_5H_4N-S-S-CH_2CH_2-NH-R$

Where R is **A**, **B** or **G**.

↓

$NH_2-(CH_2)_3-NH-CH_2-CH(OCH_2CONH-CH_2CH_2-S-S-CH_2CH_2-NH-R)-(CH_2)_2-NH-(CH_2)_3-NH_2$

Where R is **A** (**Xia**), **B** (**Xib**) or **G** (**Xic**).

Dissolve 1 mmol of **VII** in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-e-[3-(dimethylamino)propyl)carbodiimide and stir 2 hr, then add 1.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature. This solution is added dropwise to 5 mmol of 1,6-diaminohexane in 20 mL dry dimethylformamide, and stirring continued for an additional 24 hr. Remove the solvent *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid (**XIV**). Next combine 1 mmol **XIV** in dry 10 mL benzene with 1.1 mmol succinimidyl 3(2-pyridylthio)propionate, stir for 2 hr at room temperature, and then remove the solvent I *in vacuo*. The resultant product is **XV**.

$$\text{VII} \quad + \quad H_2N\text{-}(CH_2)_6\text{-}NH_2$$

$$\downarrow$$

$$Z\text{-}NH\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{OCH_2CONH\text{-}CH_2CH_2CH_2CH_2CH_2CH_2\text{-}NH_2}{\underset{|}{CH}}\text{-}(CH_2)_2\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}NH\text{-}Z \qquad \textbf{(XIV)}$$

$$\downarrow \quad \text{succinimidyl 3(2-pyridyldithio)propionate}$$

$$Z\text{-}NH\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}C_5H_4N}{\underset{|}{CH}}\text{-}(CH_2)_2\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}NH\text{-}Z \qquad \textbf{(XV)}$$

Dissolve 1 mmol **XV**, in 20 mL glacial acetic acid containing 30% Hbr and stir overnight at room temperature in the dark under N_2. Add 30 mL diethyl ether to precipitate the product. Wash the product until the odor of acetic acid is gone. Dissolve the solid in oxygen-free 0.1 M NH_4OH. The solution is applied to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product is separated from unreacted starting materials by a gradient of 20 to 80% acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product:

$$NH_2\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}C_5H_4N}{\underset{|}{CH}}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2 \qquad \textbf{(XVI)}$$

To a stirred solution of 10 mg of Fab'-SH in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 Mm **XVI** in ethanol dropwise. After 60 min. dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.

$$NH_2\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}Fab'}{\underset{|}{CH}}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2 \qquad \textbf{(XVIIg)}$$

Combine 2 mmol **IX**, 2 mmol 4-pyrrolidinopyridine and 3 mmol succinic anhydride in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid. This is followed with DCCI, *in situ* coupling with resin bound protected peptide, Pep2-COOH, using standard solid phase synthetic techniques followed by deprotection and release from the resin to yield **XIXh**.

IX + succinic anhydride

↓

$$\mathrm{Z{-}NH{-}(CH_2)_3{-}\underset{}{\overset{Z}{N}}{-}CH_2{-}\underset{OCH_2CONH{-}CH_2CH_2{-}S{-}S{-}CH_2CH_2{-}NHCO{-}CH_2CH_2{-}COOH}{CH}{-}(CH_2)_2{-}\overset{Z}{N}{-}(CH_2)_3{-}NH{-}Z} \qquad \mathrm{(XVIII)}$$

↓

$$\mathrm{NH_2{-}(CH_2)_3{-}NH{-}CH_2{-}\underset{OCH_2CONH{-}CH_2CH_2{-}S{-}S{-}CH_2CH_2{-}NHCO{-}CH_2CH_2{-}CONH{-}Pep2{-}COOH}{CH}{-}(CH_2)_2{-}NH{-}(CH_2)_3{-}NH_2} \qquad \mathrm{(XIXh)}$$

Dissolve 1 mmol of **VII** in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carabodiimide and stir 2 hr, then add 1.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature. This solution is added dropwise to 5 mmol of methyl 6-amonohexanoate in 20 mL dry dimethylformamide, and stirring continued for an additional 24 hr. Remove the solvent *in vacuo*. separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product (**XX**) as an amorphous solid.

VII + $\mathrm{H_2N{-}(CH_2)_5{-}COOCH_3}$

↓

$$\mathrm{Z{-}NH{-}(CH_2)_3{-}\overset{Z}{N}{-}CH_2{-}\underset{OCH_2CONH{-}(CH_2)_5{-}COOCH_3}{CH}{-}(CH_2)_2{-}\overset{Z}{N}{-}(CH_2)_3{-}NH{-}Z} \quad \mathrm{(XX)} \quad + \ \mathrm{OH^-}$$

Dissolve 2 mmol XX in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separate runnel, to which 2 mmol of HCl, 5 mL of water and 25 mL of benzene is added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product (**XXI**) as an amorphous solid. Next, couple **XXI** to the amino terminal of the peptide on the support using standard solid phase peptide methods.

```
            Z                      Z
            |                      |
Z-NH-(CH2)3-N-CH2-CH-(CH2)2-N-(CH2)3-NH-Z     (XXI)
                  |
                  OCH2CONH-(CH2)5-COOH
```

1,4,9,12-tetrabenzyloxycarbonyl-1,12-diamino-6-[N(5'-carboxypentyl)aminocarbonylmethoxy]-4,9-diazadodecane

↓

```
NH2-(CH2)3-NH-CH2-CH-(CH2)2-NH-(CH2)3-NH2
                  |
                  OCH2CONH-(CH2)5-CONH-R-CONH2
```

Where R is: Pep3 (**XXIIi**), Pep4 (**XXIIj**), Pep5 (**XXIIk**) or Pep6 (**XXIIl**)

Example 3

Further Tetracationic DNA Binding Templates

The overall schematic flow chart of the synthesis of these compounds is shown in Figure 6. The chemical pathway of synthesis is shown below.

A. After derivatization of the ε-N-lys with succinic anhydride it is coupled to the ligands shown below:

$H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-R$

Where R is **A**, **B**, **G** or **H**.

deprotection and release from the resin yields

```
H2N-tyr-lys-Pep11-CONH2
        |
        HN-CO(CH2)2-COHN-CH2CH2-S-S-CH2CH2-NH-R
```

Where R is **A** (**XXIIIa**), **B** (**XXIIIb**), **G** (**XXIIIc**) or **H** (**XXIV**).

Following the procedures described for the synthesis of XII and **XIII** but substituting **XXIV** for **XI** yields **XXV** and **XXVI**.

XXIV + dithiothreitol

↓

```
H2N-tyr-lys-Pep11-CONH2
        |
        HN-CO(CH2)2-COHN-CH2CH2-SH                  (XXV)
```

↓ $C_5H_4N-S-S-CH_2CH_2-CONH-R$; Where R is **D**, **E** or **F**.

NH_2-tyr-lys-Peb11-$CONH_2$
|
NHCO-CH_2-CH_2-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-R

Where R is D (**XXVId**), E (**XXVIe**) or F (**XXVIf**).

After derivatization of the ϵ-N-succinyl-lys with H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-t-BOC and deblocking, the ligand Pep2 is synthesized on the resin using standard solid phase techniques. Deblocking and cleavage from the resin yields **XXVIIh**.

H_2N-tyr-lys-Pep11-$CONH_2$ (**XXVIIh**)
|
$(CH_2)_4$NHCO-$(CH_2)_2$-CONH-$(CH_2)_2$-S-S-$(CH_2)_2$-CONH-Pep2-COOH

The resin bound lys-ϵ-NH-CO$(CH_2)_5NH_2$ intermediate was coupled with succinimidyl 3(2-pyridyldithio)propionate and then deprotected and cleaved to yield **XXVIII**.

H_2N-tyr-lys-Pep11-$CONH_2$ (**XXVIII**)
|
$(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-CH_2CH_2-S-S-C_5H_4N

To a stirred solution 10 mg of FAB'-SH in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 mM **XVI** in PBS, pH 7.4, dropwise. After 60 min. dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.

H_2N-tyr-lys-Pep11-$CONH_2$
|
$(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-CH_2CH_2-S-S-Fab' (**XXIXg**)

The nuclear localization sequence was added by standard solid phase synthetic methods to the lys-ϵ-NH-CO$(CH_2)_5NH_2$ intermediate of the resin bound protected peptide for carboxyl to amino orientation or to the N-succinyl derivative of ϵ-N-lys for amino to carboxyl orientation. Deprotection and release from the resin yields:

H_2N-tyr-lys-Pep11-$CONH_2$
|
$(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-R-NH_2

Where R is Pep7 (**XXXi**), Pep8 (**XXXj**), Pep9 (**XXXk**) or Pep10 (**XXXl**)

or

H_2N-tyr-lys-Pep11-$CONH_2$
|
$(CH_2)_4$NHCO-$(CH_2)_2$-CONH-R-$CONH_2$

Where R is Pep3 (**XXXIi**), Pep4 (**XXXIj**), Pep5 (**XXXIk**) or Pep6 (**XXXIl**)

B. PARENT COMPOUND: N-(ligand moiety)-HN-tyr-lys-lys-ala-lys-ala-lys-ala-lys-$CONH_2$, prepared by standard solid phase peptide synthesis. It is obvious that any amino acid polymer, such as H_2N-$(lys)_n$-COOH, H_2N-$(arg\text{-}ala)_n$-COOH, histones, and other DNA binding cationic polypeptides and proteins which form an α-helix, could be substituted for the lys-ala template. The -HN-$(lys\text{-}ala)_n$-CO unit can be extended from 4 to more than 100. The sequence position of the residue bearing the spacer-ligand moiety can be either amino-terminal or carboxyl-terminal. In another embodiment, the ligand-spacer moiety is linked through a disulfide bond to cys as either the N-terminal or C-terminal residue.

The resin bound protected peptide containing a deblocked α-amino-tyr moiety is the synthetic intermediate for preparation of templates for reductive release of a plasma membrane receptor ligand. After derivatization of the α-amino-tyr with succinic anhydride, the following ligands are coupled to the resin bound protected peptide.

H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-R

where R = γ-amide of the glutamyl moiety of folic acid (A)
= biotin (B)
= lipoic acid (G)
= H

Deprotection and release from the resin gives:

tyr-lys-lys-ala-lys-ala-lys-ala-lys-$CONH_2$
|
HN-CO$(CH_2)_2$-COHN-CH_2CH_2-S-S-CH_2CH_2-NH-R

whereR = γ-amide of the glutamyl moiety of folic acid (XXIIIa)
= biotin (XXIIIb)
= lipoic acid (XXIIIc)
= H

Example 4

Synthesis of Hexacationic DNA Binding Template

A schematic flow chart of the synthesis of these compounds is shown in Figure 7. The chemical pathway of synthesis is shown below.

Dissolve 2 mmol of succinic monoamide in 2 mL dry DMF, add 4.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carbodiimide and stir 2 hr.

Then add 2.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature. Combine this in dropwise fashion to 1 mmol (S)-hydroxyspermine (IV) in 2 mL dry DMF. After stirring overnight at room temperature, remove the solvent *in vacuo*, dissolve in water, and apply the solution to a cation exchange resin. The product is isolated by a gradient to 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain **XXXII**.

Dissolve 2 mmol **XXXII** in 5 mL dry toluene and add 10 mmol sodium bis(2-methoxyethoxy)aluminum hydride in toluene in small aliquots over 30 min. After 2 hr, add 10 mL ethyl acetate, then remove the solvents *in vacuo*. Dissolve the solids in water, adjust the pH to 3 with HCl and apply to a cation exchange resin. The product is isolated by a gradient to 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain the product.

$$H_2N-(CH_2)_3-NH-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-(CH_2)_2-NH-(CH_2)_3-NH_2 \quad \textbf{(IV)}$$

$$+ \quad H_2NCOCH_2CH_2COOH$$

$$\downarrow$$

$$H_2NCOCH_2CH_2CONH-(CH_2)_3-NH-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-(CH_2)_2-NH-(CH_2)_3-NHCOCH_2CH_2CONH_2 \quad \textbf{(XXXII)}$$

$$\downarrow \text{ Red-Al}$$

$$H_2N(CH_2)_4-NH-(CH_2)_3-NH-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_4-NH_2 \quad \textbf{(XXXIII)}$$

It is obvious to one skilled in the art that homologs with additional $-(CH_2)_4-NH_2$, or $-(CH_2)_3-NH_2$ units can be made by repeating the reactions using **XXXIII** in lieu of **IV** with $H_2NCOCH_2CH_2COOH$ or $CH_2=CHCN$.

In reactions similar to the above reaction where **IV** is converted to **VII**, **XXXIII** can be converted to **XXVI**.

$$\textbf{XXXIII} \quad + \quad Z_2O$$

$$\downarrow$$

$$Z-HN-(CH_2)_4-\overset{\displaystyle Z}{\overset{|}{N}}-(CH_2)_3-\overset{\displaystyle Z}{\overset{|}{N}}-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-(CH_2)_2-\overset{\displaystyle Z}{\overset{|}{N}}-(CH_2)_3-\overset{\displaystyle Z}{\overset{|}{N}}-(CH_2)_4-NH-Z \quad \textbf{(XXXIV)}$$

$$\downarrow \; BrCH_2-COOCH_3, \; NaOCH_3$$

$$Z\text{-}HN\text{-}(CH_2)_4\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{\underset{OCH_2COOCH_3}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_4\text{-}NH\text{-}Z \quad \textbf{(XXXV)} \quad + \quad OH^-$$

$$\downarrow$$

$$Z\text{-}HN\text{-}(CH_2)_4\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{\underset{OCH_2COOH}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_4\text{-}NH\text{-}Z \quad \textbf{(XXXVI)}$$

1,5,9,14,18,22-hexabenzyloxycarbonyl-1,22-diamino-11-carboxymethoxy-5,9,14,18-tetraazadocosane

In reactions similar to the above reactions where **VII** is converted to the **VII** and **XI** series, **XXXVI** can be converted to the **XXXVII** and **XXXIX** series.

XXXVI + $H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$

Where R is **A**, **B**, **G** or **H**.

$$\downarrow$$

$$Z\text{-}HN\text{-}(CH_2)_4\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{\underset{OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}\overset{Z}{\overset{|}{N}}\text{-}(CH_2)_4\text{-}NH\text{-}Z$$

Where R is **A** (**XXXVIIa**), **B** (**XXXVIIb**), **G** (**XXXVIIc**) or **H** (**XXXVIII**).

$$\downarrow \quad HBr,\ CH_3COOH$$

$$H_2N\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{\underset{OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R}{|}}{CH}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2$$

Where R is **A** (**XXXIXa**), **B** (**XXXIXb**), **G** (**XXXIXc**) or **H** (**XL**).

In reactions similar to the above for the conversion of **XI** to the **XIII** series **XL** is converted to the **XLII** series.

XL + dithiothreitol

$$\downarrow$$

$$H_2N\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}\underset{\underset{OCH_2CONH\text{-}CH_2CH_2\text{-}SH}{|}}{CH}\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2 \quad \textbf{(XLI)}$$

$\downarrow$ $C_5H_4N\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}CONH\text{-}R$; Where R is **D**, **E** or **F**.

$$NH_2-(CH_2)_3-NH-CH_2-\underset{\displaystyle |\atop OCH_2CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-R}{CH}-(CH_2)_2-NH-(CH_2)_3-NH_2$$

Where R is **D** (**XLIId**), **E** (**XLIIe**) or **F** (**XLIIf**)

In reactions similar to the above conversion of **XII** to the **XI** series, **XLI** is converted to the **XXXIX** series.

$$\mathbf{XLI} \quad + \quad C_5H_4N-S-S-CH_2CH_2-NH-R$$

Where R is **A**, **B** or **G**.

$$\downarrow$$

$$H_2N-(CH_2)_4-NH-(CH_2)_3-NH-CH_2-\underset{\displaystyle |\atop OCH_2CONH-CH_2CH_2-S-S-CH_2CH_2-NH-R}{CH}-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_4-NH_2$$

Where R is **A** (**XXXIXa**), **B** (**XXXIXb**) or **G** (**XXXIXc**).

In reactions similar to the above conversion of **VII** to **XIV** and **XVIIg**, **XXXVI** is converted to **XLIV** and **XLVIg**.

$$\mathbf{XXXVI} \quad + \quad H_2N-(CH_2)_6-NH_2$$

$$\downarrow$$

$$Z-HN-(CH_2)_4-\overset{Z\atop |}{N}-(CH_2)_3-\overset{Z\atop |}{N}-CH_2-\underset{\displaystyle |\atop OCH_2CONH-CH_2CH_2CH_2CH_2CH_2CH_2-NH_2}{CH}-(CH_2)_2-\overset{Z\atop |}{N}-(CH_2)_3-\overset{Z\atop |}{N}-(CH_2)_4-NH-Z \qquad (\mathbf{XLIII})$$

$\downarrow$ + succinimidyl 3(2-pyridyldithio)propionate

$$Z-HN-(CH_2)_4-\overset{Z\atop |}{N}-(CH_2)_3-\overset{Z\atop |}{N}-CH_2-\underset{\displaystyle |\atop OCH_2CONH-(CH_2)_6-NHCO-CH_2CH_2-S-S-C_5H_4N}{CH}-(CH_2)_2-\overset{Z\atop |}{N}-(CH_2)_3-\overset{Z\atop |}{N}-(CH_2)_4-NH-Z \qquad (\mathbf{XLIV})$$

$\downarrow$ + HBr, CH_3COOH

$$H_2N-(CH_2)_4-NH-(CH_2)_3-NH-CH_2-\underset{\displaystyle |\atop OCH_2CONH-(CH_2)_6-NHCO-CH_2CH_2-S-S-C_5H_4N}{CH}-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_4-NH_2 \qquad (\mathbf{XLV})$$

$\downarrow$ + Fab'-SH

$$H_2N-(CH_2)_4-NH-(CH_2)_3-NH-CH_2-\underset{\displaystyle |\atop OCH_2CONH-(CH_2)_6-NHCO-CH_2CH_2-S-S-Fab'}{CH}-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_4-NH_2 \qquad (\mathbf{XLVIg})$$

In reactions similar to the above conversion of **IX** to **XIXh**, **XXXVIII** is converted to **XLVIIIh**.

XXXVIII + succinic anhydride

↓

Z Z Z Z (on the four ring-chain N atoms)

$Z\text{-}HN\text{-}(CH_2)_4\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}(CH_2)_4\text{-}NH\text{-}Z$ **(XLVII)**

$\quad\quad | \; OCH_2CONH\text{-}CH_2CH_2\text{-}S$

$\quad\quad\quad\quad\quad\quad\quad\quad | $

$HOOC\text{-}CH_2CH_2\text{-}COHN\text{-}CH_2CH_2\text{-}S$

$H_2N\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2$

$\quad\quad | \; OCH_2CONH\text{-}(CH_2)_2S$ **(XLVIIIh)**

$\quad\quad\quad\quad\quad\quad\quad\quad | $

$HOOC\text{-}Pep2\text{-}HNOC\text{-}CH_2CH_2\text{-}COHN\text{-}CH_2CH_2\text{-}S$

In reactions similar to the above conversion of **VII** to **XXII** series, **XXXVI** is converted to the **LI** series.

XXXVI + $H_2N\text{-}(CH_2)_5\text{-}COOCH_3$

↓

$Z\text{-}HN\text{-}(CH_2)_4\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}(CH_2)_4\text{-}NH\text{-}Z$ **(XLIX)**

$\quad\quad | \; OCH_2CONH\text{-}(CH_2)_5\text{-}COOCH_3$ + OH^-

↓

$Z\text{-}HN\text{-}(CH_2)_4\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N(Z)\text{-}(CH_2)_3\text{-}N(Z)\text{-}(CH_2)_4\text{-}NH\text{-}Z$ **(L)**

$\quad\quad | \; OCH_2CONH\text{-}(CH_2)_5\text{-}COOH$

1,5,9,14,18,22-hexabenzyloxycarbonyl-1,22-diamino-11-[N(5'-carboxypentyl)carbonylmethoxy-5,9,14,18-tetraazadocosane

$NH_2\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$

$\quad\quad | \; OCH_2CONH\text{-}(CH_2)_5\text{-}CONH\text{-}R\text{-}CONH_2$

Where R is: Pep3 (**LIi**), Pep4 (**LIj**), Pep5 (**LIk**) or Pep6 (**LIl**)

Example 5

Intercalating hexacationic DNA Binding Templates

A schematic flow chart for the synthesis of these compounds is shown in Figure 8. The chemical pathway of synthesis is shown below.

Combine 2 mmol III (resolved S enantiomer), 4 mmol 4-pyrrolidinopyridine and 4.1 mmol benzyloxycarbonyl anhydride in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product by solid phase extraction on phenyl-silica

and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product (**LII**) as an amorphous solid.

III (resolved S enantiomer) + t-BOC$_2$O

↓

```
           t-BOC                 t-BOC
             |                     |
CN-(CH2)2-N-CH2-CH-(CH2)2-N-(CH2)2-CN                    (LII)
                 |
                 OH        + BrCH2-COOCH3, NaOCH3
```

Add 4 mL of dry benzene to 1 mmol K_2CO_3 and 2 mmol 18-crown-6 and stir for 20 min. Add 2 mmol **LIII** in 4 mL of dry benzene, followed by 2 mmol methyl bromoacetate in 2 mL benzene. After 4 hr., add 25 mL of water and extract with 3 portions of 25 mL benzene. Remove the solvent *in vacuo* to obtain the product:

```
           t-BOC                 t-BOC
             |                     |
CN-(CH2)2-N-CH2-CH-(CH2)2-N-(CH2)2-CN                    (LIII)
                 |
                 OCH2-COOCH3
```

↓ H_2, Raney Ni

```
            t-BOC                 t-BOC
              |                     |
H2N-(CH2)3-N-CH2-CH-(CH2)2-N-(CH2)3-NH2                  (LIV)
                  |
                  OCH2-COOCH3
```

Dissolve 5 mmol **LIV** in 10 mL dry pyridine containing 0.1 mmol dimethylaminopyridine and 15 mmol triethylamine. Add dropwise 11 mmol 6,9-dichloro-2-methoxyacridine on any DNA binding dye that reacts specifically with amino group in 10 mL dry pyridine to the stirred solution. Stir for over 1 hr at room temperature. The solvents are removed *in vacuo*, and the mixture is redissolved in acetonitrile for solid phase extraction on phenyl-silica and elution with a linear gradient of acetonitrile to 50% in hexane. The appropriate fractions were pooled and the solvent evaporated to obtain the product as an amorphous solid.

```
                         t-BOC                 t-BOC
                           |                     |
C14H9ClNO-HN-(CH2)3-N-CH2-CH-(CH2)2-N-(CH2)3-NH-C14H9ClNO     (LV)
                               |
                               OCH2-COOCH3  + OH-
```

Dissolve 2 mmol LV in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 2 mmol of HCl, 5 mL of water and 25 mL of benzene is added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*. Dissolve in dry DMF for standard solid phase peptide synthesis.

$$\begin{array}{c} \text{t-BOC} \qquad\qquad\qquad \text{t-BOC} \\ C_{14}H_9ClNO\text{-}HN\text{-}(CH_2)_3\text{-}N(\text{t-BOC})\text{-}CH_2\text{-}CH(OCH_2\text{-}COOH)\text{-}(CH_2)_2\text{-}N(\text{t-BOC})\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO \end{array} \qquad \textbf{(LVI)}$$

1,12-N,N'-di(2-methoxy-6-chloroacridinyl)amino-4,9-di-*t*-butyoxycarbonyl-6-carboxymethoxy-4,9-diaza-dodecane

In reactions similar to the conversions in Example 2, the following products are obtained. One skilled in the art will recognize that the reaction conditions are similar, but that the starting material and end product will be different.

LVI + $H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$

↓ Where R is **A**, **B**, **G** or **H**.

$$C_{14}H_9ClNO\text{-}HN\text{-}(CH_2)_3\text{-}N(\text{t-BOC})\text{-}CH_2\text{-}CH(OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R)\text{-}(CH_2)_2\text{-}N(\text{t-BOC})\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$$

Where R is **A** (**LVIIa**), **B** (**LVIIb**), **G** (**LVIIc**) or H (**LVIII**).

↓ HBr, CH_3COOH

$$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH(OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R)\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$$

Where R is **A** (**LIXa**), **B** (**LIXb**), **G** (**LIXc**) or H (**LX**).

(b) **LX** + dithiothreitol

↓

$$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH(OCH_2CONH\text{-}CH_2CH_2\text{-}SH)\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO \qquad \textbf{(LXI)}$$

↓ $C_5H_4N\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}CONH\text{-}R$; Where R is D, E or F.

$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$

| (on CH) $OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}CONH\text{-}R$

Where R is **D** (**LXIId**), **E** (**LXIIe**) or **F** (**LXIIf**).

LXI + $C_5H_4N\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$; Where R is **A**, **B** or **G**.

↓

$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$

| (on CH) $OCH_2CONH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$

Where R is **A** (**LIXa**) **B** (**LIXb**) or **G** (**LIXc**).

LVI + $H_2N\text{-}(CH_2)_6\text{-}NH_2$

↓

$C_{14}H_9ClNO\text{-}HN\text{-}(CH_2)_3\text{-}N(t\text{-}BOC)\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N(t\text{-}BOC)\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$

| (on CH) $OCH_2CONH\text{-}(CH_2)_6\text{-}NH_2$ (**LXIII**)

↓ succinimidyl 3(2-pyridyldithio)propionate

$C_{14}H_9ClNO\text{-}HN\text{-}(CH_2)_3\text{-}N(t\text{-}BOC)\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N(t\text{-}BOC)\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$ (**LXIV**)

| (on CH) $OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}C_5H_4N$

↓ + HBr, CH_3COOH

$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$ (**LXV**)

| (on CH) $OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}C_5H_4N$

↓ + Fab'-SH

$C_{14}H_9ClNO\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}C_{14}H_9ClNO$

| (on CH) $OCH_2CONH\text{-}(CH_2)_6\text{-}NHCO\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}Fab'$ (**LXVIg**)

LVI + succinic anhydride

↓

t-BOC t-BOC

$C_{14}H_9ClNO-HN-(CH_2)_3-N-CH_2-CH-(CH_2)_2-N-(CH_2)_3-NH-C_{14}H_9ClNO$ **(LXVII)**

$OCH_2CONH-CH_2CH_2-S$

$HOOC-CH_2CH_2-OCHN-CH_2CH_2-S$

↓

$C_{14}H_9ClNO-NH-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NH-C_{14}H_9ClNO$ **(LXVIIIh)**

$O-CH_2-CONH-(CH_2)_2-S$

$HOOC-Pep2-HNOC-CH_2CH_2-HNOC-CH_2CH_2-S$

LVI + $H_2N(CH_2)_5-COOCH_3$

↓

t-BOC t-BOC

$C_{14}H_9ClNO-HN-(CH_2)_3-N-CH_2-CH-(CH_2)_2-N-(CH_2)_3-NH-C_{14}H_9ClNO$ **(LXIX)**

$OCH_2-CONH(CH_2)_5-COOCH_3$ + OH^-

↓

t-BOC t-BOC

$C_{14}H_9ClNO-HN-(CH_2)_3-N-CH_2-CH-(CH_2)_2-N-(CH_2)_3-NH-C_{14}H_9ClNO$ **(LXX)**

$OCH_2-CONH(CH_2)_5-COOH$

1,12-N,N'-di(2-methoxy-6-chloroacridinyl)amino-4,9-di-t-butyoxycarbonyl-6-[N(5'-carboxypentyl)aminocarbonylmethoxy]-4,9-diaza-dodecane

↓

$C_{14}H_9ClNO-NH-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NH-C_{14}H_9ClNO$

$OCH_2CONH-(CH_2)_5-CONH-R-CONH_2$

Where R is Pep3 **(LXXIi)**, Pep4 **(LXXIj)**, Pep5 **(LXXIk)** or Pep6 **(LXXIl)**.

Example 6

Further Intercalating hexacationic DNA Binding Template

A schematic flow chart for the synthesis of these compounds is shown in Fig. 9. The chemical pathway of synthesis is shown below.

In reactions similar to the conversions in Example 3, the following products are obtained. One skilled in the art will recognize

that the reaction conditions are similar, but that the starting material and end products will be different.

After derivatization of the ε-N-lys with succinic anhydride, the following ligands are coupled to the resin bound protected peptide.

$H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-R$

Where R is **A**, **B**, **G** or **H**.

deprotection and release from the resin yields:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO-NH-tyr-lys-Pep11-CONH-CH_2CH_2-CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad HN-CO(CH_2)_2-COHN-CH_2CH_2-S-S-CH_2CH_2-NH-R \end{array}$$

Where R is **A** (**LXXIIa**), **B** (**LXXIIb**), **G** (**LXXIIc**) or **H** (**LXXIII**).

LXXIII + dithiothreitol

↓

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO-NH-tyr-lys-Pep11-CONH-CH_2CH_2-CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad HN-CO(CH_2)_2-COHN-CH_2CH_2-SH \end{array}$$

(**LXXIV**)

↓ $C_5H_4N-S-S-CH_2CH_2-CONH-R$; Where R is D, E or F.

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C\ \ {}_9ClNO-NH-tyr-lys-Pep11-CON^4H-CH_2CH_2-CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad CONH-CO-(CH_2)_2-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-R \end{array}$$

Where R is **D** (**LXXVd**), **E** (**LXXVe**) or **F** (**LXXVf**).

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO-NH-tyr-lys-Pep11-CON^4H-CH_2CH_2.CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad (CH_2)_4-HNCO-(CH_2)_2.CONH-(CH_2)_2-S \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\ \ | \\ \qquad\qquad\qquad\qquad\qquad\quad HOOC-Pep2-HNOC-CH_2CH_2-S \end{array}$$

(**LXXVIh**)

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO-NH-tyr-lys-Pep11-CONH-CH_2CH_2-CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad (CH_2)_4NHCO-(CH_2)_5-NHCO-CH_2CH_2-S-S-C_5H_4N \end{array}$$

(**LXXVII**)

↓ + Fab'-SH

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO-NH-tyr-lys-Pep11-CONH-CH_2CH_2-CH-NH-C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad\qquad\quad (CH_2)_4NHCO-(CH_2)_5-NHCO-CH_2CH_2-S-S-Fab' \end{array}$$

(**LXXVIIIg**)

The addition of the nuclear localization sequence yields:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO\text{-}NH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}CONH\text{-}CH_2CH_2\text{-}CH\text{-}NH\text{-}C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\quad (CH_2)_4NHCO\text{-}(CH_2)_5\text{-}NHCO\text{-}R\text{-}NH_2 \end{array}$$

where R is Pep7 (**LXXIXi**), Pep8 (**LXXIXj**), Pep9, (**LXXIXk**) or Pep10 (**LXXIXl**).

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CONH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ C_{14}H_9ClNO\text{-}NH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}CONH\text{-}CH_2CH_2\text{-}CH\text{-}NH\text{-}C_{14}H_9ClNO \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\quad (CH_2)_4NHCO\text{-}(CH_2)_2\text{-}CONH\text{-}R\text{-}CONH_2 \end{array}$$

where R is Pep3 (**LXXXi**), Pep4 (**LXXXj**), Pep5 (**LXXXk**) or Pep6 (**LXXXl**).

Example 7

Dimeric Octacationic DNA Binding Templates

A schematic flow chart for the synthesis of these compounds is shown in Figure 10. The chemical pathway of synthesis is shown below.

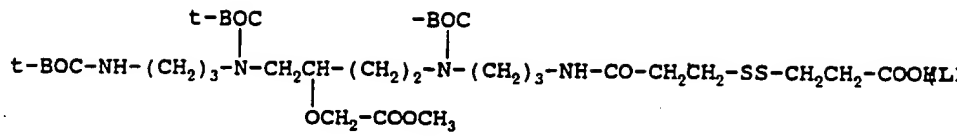
Combine 2 mmol **LIV**, 1 mmol 4-pyrrolidinopyridine and 1.0 mmol benzyloxycarbonyl anhydride in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate, 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the produce (**LXXXI**) as an amorphous solid.

LIV + 0.5 mole t-BOC_2O

↓

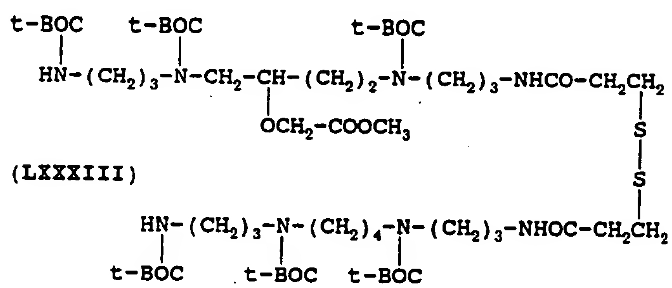
$$\begin{array}{l} \qquad\qquad\quad t\text{-}BOC \qquad\qquad\qquad\quad t\text{-}BOC \\ \qquad\qquad\qquad | \qquad\qquad\qquad\qquad\quad | \\ t\text{-}BOC\text{-}HN\text{-}(CH_2)_3\text{-}N\text{-}CH_2\text{-}CH\text{-}(CH_2)_2\text{-}N\text{-}(CH_2)_3\text{-}NH_2 \qquad (\mathbf{LXXXI}) \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\quad OCH_2\text{-}COOCH_3 \end{array}$$

Combine 2 mmol **LXXXI**, 2 mmol 4-pyrrolidinopyridine and 3 mmol bis-(3-carboxyethyl)dithiol in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product (**LXXXII**) as an amorphous solid.



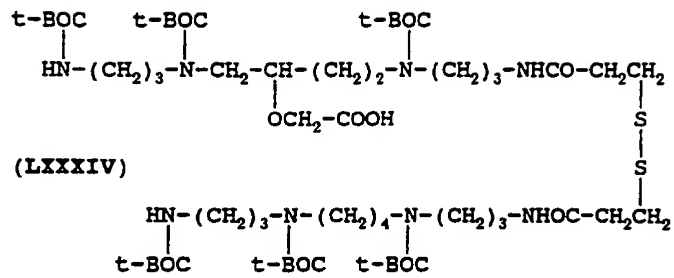
(LXXXII)

Dissolve 2 mmol **LXXXII** in 2 mL dry DMF, add 4.0 mmol 1-ethyl-3-[3-(dimethyl-amino)propyl)carbodiimide and stir 2 hr, then add 2.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature, then combine with 2 mmol 3-[(3''-N-t-BOC-aminopropyl)-4'-N-t-BOC-aminobutyl]-N-t-BOC-aminopropylamine in 21 mL dry DMF. After stirring overnight at room temperature, remove the solvent *in vacuo*, separate the product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate, 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product **(LXXXIII)** as an amorphous solid.



(LXXXIII)

Dissolve 2 mmol **LXXXIII** in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 2 mmol of HCI, 5 mL of water and 25 mL of benzene is added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product **(LXXXIV)** as an amorphous solid.



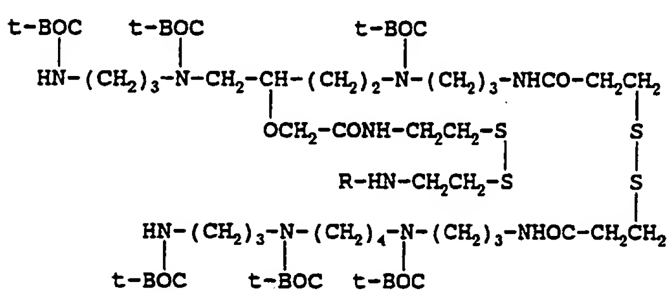
(LXXXIV)

Dissolve 1 mmol of **LXXXIV** in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carbodiimide and stir 2 hrs., then add 1.1 mmol n-hydroxysuccinimide and continue stirring for another 6 hrs. at room temperature. This solution is added dropwise to 3 mmol of $H_2N-CH_2CH_2-NH-R$ (where R = **A**, **B**, **G** or H) in 20 mL dry dimethylformamide, and stirring continued for an additional 24 hrs. Remove the solvent *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

LXXXIV + $H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-R$

where R is **A**, **B**, **G** or H.

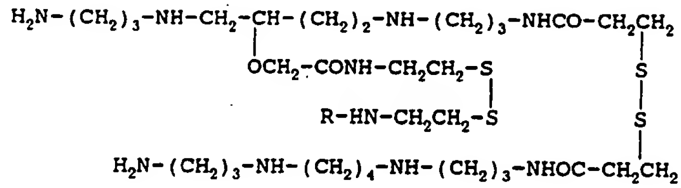
↓



where R is **A** (**LXXXVa**), **B** (**LXXXVb**), **G** (**LXXXVc**) or H (**LXXXVI**).

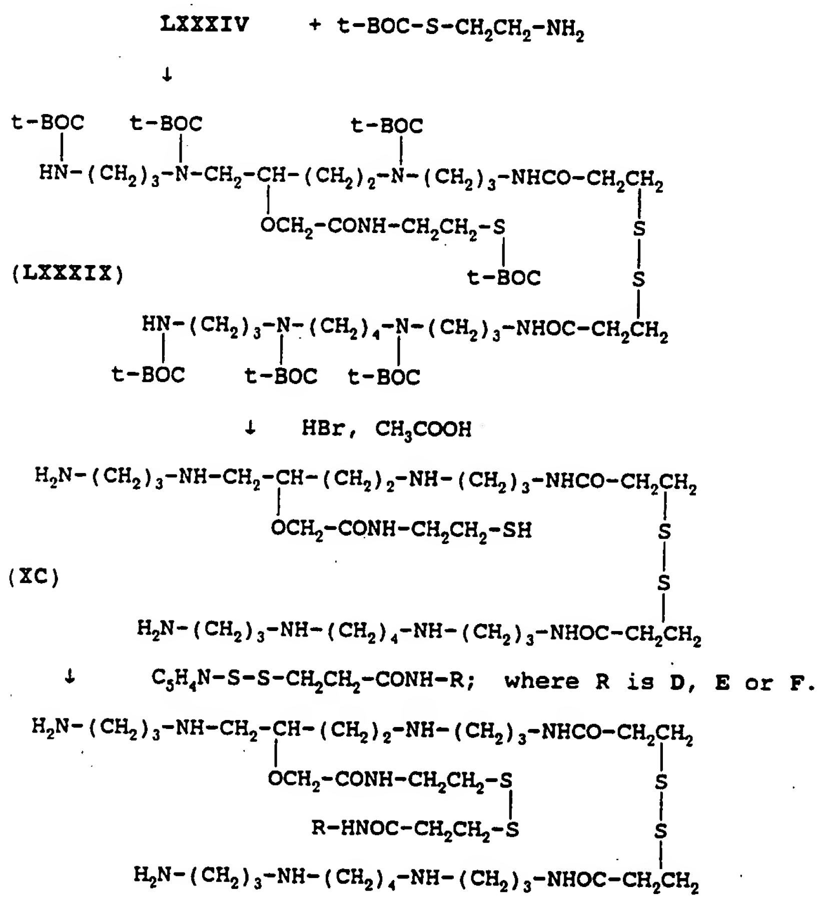
Dissolve **LXXXVa**, 1 mmol, in 29 mL glacial acetic acid containing 30% HBr and stir overnight at room temperature in the dark under N_2. Add 30 mL diethyl ether to precipitate the product. Wash the product until the odor of acetic acid is gone. Dissolve the solid in oxygen-free 0.1 M NH_4OH. The solution is applied to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product is separated from unreacted starting materials by a

gradient of 20 to 80% acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product:



where R is **A** (**LXXXVIIa**), **B** (**LXXXVIIb**), **G** (**LXXXVIIc**) or H (**LXXXVIII**).

Use the same procedures as for **LXXXVa-LXXXVc**, except substitute S-t-BOC-mercaptoethylamine for $H_2N-CH_2CH_2-SS-CH_2CH_2-NH-R$. Then use the same procedures as for **LXXXVIIa-LXXXVIIc**.



(**LXXXIX**)

(**XC**)

where R is **D** (**XCId**), **E** (**XCIe**) or **F** (**XCIf**)

Dissolve 1 mmol **xc** in 2 mL phosphate-buffered saline, pH 7.4, and combine with 1 mmol A', also dissolved in 2 mL phosphate-buffered

saline, pH 7.4. Dilute the reaction mixture 20-fold with water, apply to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product **XIa** is separated from unreacted starting materials by a gradient of 20 to 80% acetonitrile in 0.1 M NH_4CO_3. The appropriate actions are pooled by lyophilized to obtain the product.

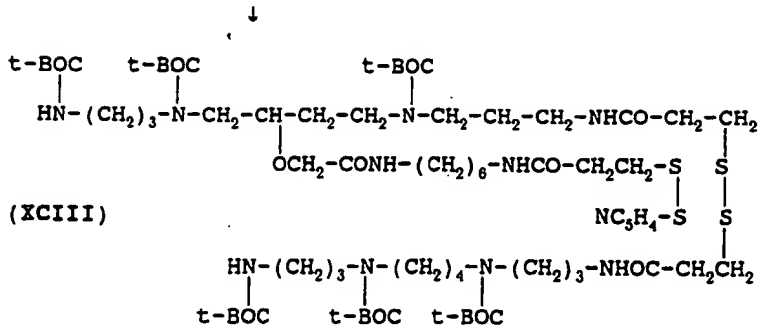
```
XC     +     C5H4N-S-S-CH2CH2-NH-R; where R is A, B or G.
       ↓
H2N-(CH2)3-NH-CH2-CH-(CH2)2-NH-(CH2)3-NHCO-CH2CH2
                  |                             |
                  OCH2-CONH-CH2CH2-S            S
                                   |            |
                         R-HN-CH2CH2-S          S
                                                |
      H2N-(CH2)3-NH-(CH2)4-NH-(CH2)3-NHOC-CH2CH2
```

where R is **A** (**LXXXVIIa**), **B** (**LXXXVIIb**) or **G** (**LXXXVIIc**).

Dissolve 1 mmol of **LXXXIV** in 2 mL dry dimethylformamide, add 3.0 mmol 1-ethyl-3-[3-(diamethylamino)propyl) carbodiimide and stir 2 hr, then add 1.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature. This solution is added dropwise to 5 mmol of 1,6-diaminohexane in 20 mL dry diamethylformamide, and stirring continued for an additional 24 hr. Remove the solvent *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate from 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

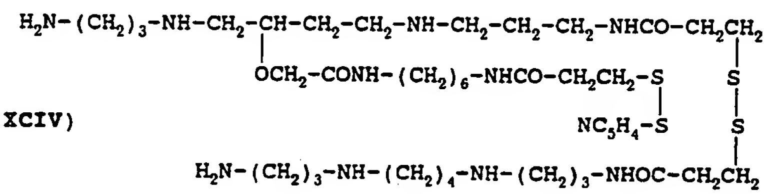
```
LXXXIV       +      H2N-(CH2)6-NH2
           ↓
t-BOC   t-BOC              t-BOC
 |        |                  |
HN-(CH2)3-N-CH2-CH-(CH2)2-N-(CH2)3-NHCO-CH2CH2
                 |                            |
                 OCH2-CONH-(CH2)6-NH2         S
                                              |
(XCII)                                        S
                                              |
     HN-(CH2)3-N-(CH2)4-N-(CH2)3-NHOC-CH2CH2
      |         |        |
    t-BOC     t-BOC    t-BOC
```

Combine 1 mmol **XCII** in dry 10 mL benzene with 1.1 mmol succinimidyl 3(2-pyridylthio) propionate, stir for 2 hr at room temperature, and then remove the solvent *in vacuo*.



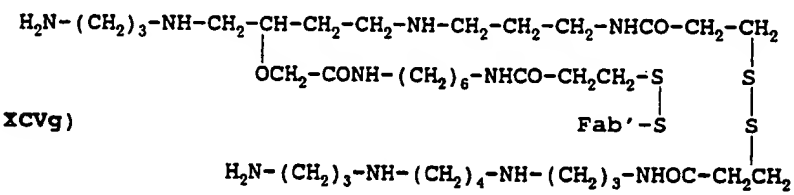
(XCIII)

Dissolve **XCIII**, 1 mmol, in 20 mL glacial acetic acid containing 30% HBr and stir overnight at room temperature in the dark under N_2. Add 30 mL diethyl ether to precipitate the product. Wash the product until the odor of acetate acid is gone. Dissolve the solid in oxygen-free 0.1 M NH_4CO_3. The solution is applied to an anion exchange resin equilibrated in degassed 0.1 M NH_4CO_3 containing 20% acetonitrile. The product is separated from unreacted starting materials by a gradient of 20 to 80% acetonitrile in 0.1 M NH_4CO_3. The appropriate fractions are pooled and lyophilized to obtain the product.



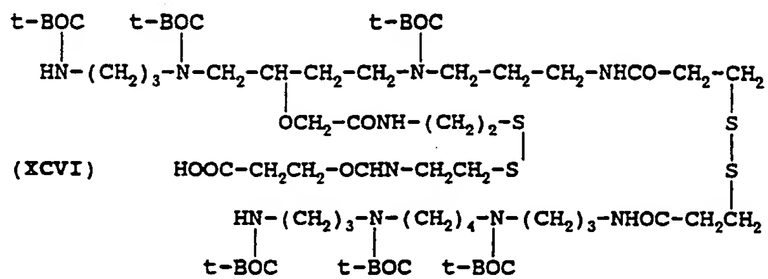
(XCIV)

To a stirred solution of 10 mg of Fab'-SH in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 mM **XCIV** in PBS, pH 7.4, dropwise. After 60 min, dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.



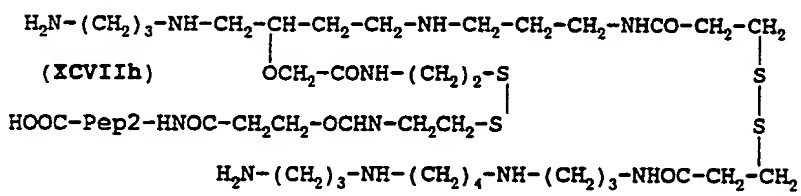
(XCVg)

Combine 2 mmol **LXXXVI**, 2 mmol 4-pyrrolidinopyridine and 3 mmol succinic anhydride in 40 mL anhydrous benzene and stir overnight at room temperature under N_2. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product (**XCVI**) as an amorphous solid.



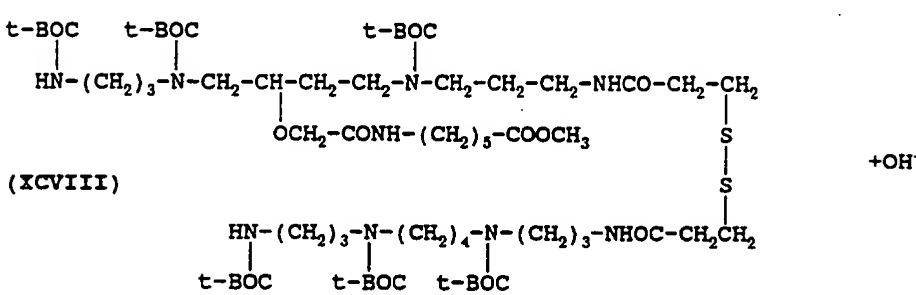
(XCVI)

Couple **XCVI** to the amino terminal of the peptide on the support using standard solid phase peptide methods, cleave from the resin and deprotect, and purify by ion exchange chromatography.



(XCVIIh)

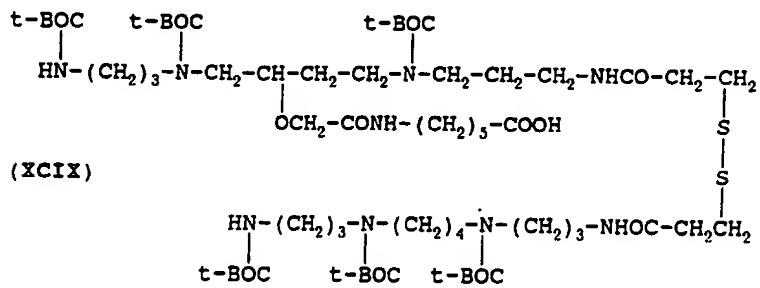
Dissolve 2 mmol **LXXXIV** in 2 mL dry DMF, add 4.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carbodiimide and stir 2 hr, then add 2.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature, then combine with 2 mmol methyl 6-aminohexanoate in 2 mL dry DMF. After stirring overnight at room temperature, remove the solvent *in vacuo*, separate the product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate, 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.



(XCVIII)

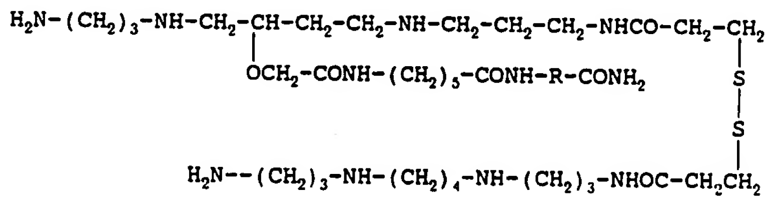
Dissolve 2 mmol **XCVIII** in 10 mL ethanol containing 2 mmol potassium hydroxide. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 2 mmol of HCl, 5 mL of water and 25 mL of benzene is added. After extraction with 3 additional portions of benzene, the combined organic phase is taken to

dryness *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 100% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.



(XCIX)

Couple **XCIX** to the amino terminal of the appropriate peptide on the support using standard solid phase peptide methods, cleave from the resin and deprotect, and purify by ion exchange chromatography.



where R is Pep3 (**XCXi**), Pep4 (**XCXj**), Pep5 (**XCXk**) or Pep6 (**XCXl**).

Example 8

Further Dimeric Octacationic DNA Binding Templates

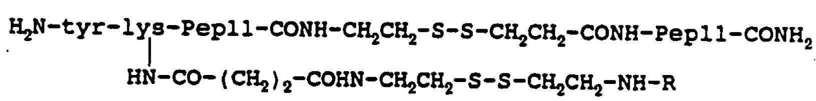
A schematic flow chart for the synthesis of these compounds is shown in Figure 11. The chemical pathways of synthesis are shown below.

After derivatization of the ε-N-Lys or α-N-Tyr with succinic anhydride the following ligands are coupled to the resin bound protective peptide:

$H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$

where R is **A**, **B** or **G**.

Deprotection and release from the resin yields:



where R is **A** (**CIa**), **B** (**CIb**) or **G** (**CIc**).

After derivatization of the ϵ-N Lys with t-BOC-S-$(CH_2)_2$-COOH, deprotection and release from the support yields:

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
|
HN-CO-CH_2CH_2-SH (CII)

To a stirred solution of 10 mg of **D** or **E** in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 mM **CII** in PBS, pH 7.4, dropwise. After 60 min. dilute the reaction mixture 20-fold with water, apply to a cation exchange column to separate the desired product from unreacted starting material and other products, using a linear gradient formed from equal volumes of water and 2.0 M HCl. The appropriate fractions are pooled and lyophilized to obtain the product. Alternatively, to a stirred solution of 10 mg of F or F' in 5 mL PBS, pH 7.4, at 4° add 0.3 mL of 10 mM **CII** in PBS, pH 7.4, dropwise. After 60 min, dialyze against 3 changes of 0.5 L PBS, pH 7.4, at 4°, each for 2 hr.

↓ + C_5H_4N-S-S-CH_2CH_2-CONH-R; where R is **D**, **E** or **F**.

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
|
HN-CO-CH_2CH_2-S-S-CH_2CH_2-CONH-R

where R is **D** (**CIIId**), **E** (**CIIIe**) or **F** (**CIIIf**).

After derivatization of the ϵ-N-succinyl-lys with H_2N-CH_2-CH_2-S-S-CH_2CH_2-NH-t-BOC and deblocking, the ligand Pep2 is synthesized on the resin using standard solid phase technique. Deblocking and cleavage from the resin yields:

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$ (CIVh)
|
$(CH_2)_4$-NHCO-$(CH_2)_2$-CONH-$(CH_2)_2$-S-S-$(CH_2)_2$-CONH-Pep2-COOH

The resin bound Lys-ϵNH-CO$(CH_2)_5$ NH_2 intermediate is coupled with succinimidyl 3(2-pyridyldithio) propionate and then deprotected and cleaved to yield:

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
|
$(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-CH_2CH_2-S-S-C_5H_4N (CV)

↓ + Fab'-SH

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
|
$(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-CH_2CH_2-S-S-Fab' (CVIg)

The addition of the nuclear localization sequence yields:

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
    |
    $(CH_2)_4$NHCO-$(CH_2)_5$-NHCO-R-NH_2

where R is Pep7 (**CVIIi**), Pep8 (**CVIIj**), Pep9 (**CVIIk**) or Pep10 (**CVIIl**).

H_2N-tyr-lys-Pep11-CONH-CH_2CH_2-S-S-CH_2CH_2-CONH-Pep11-$CONH_2$
    |
    $(CH_2)_4$NHCO-$(CH_2)_2$-CONH-R-$CONH_2$

where R is Pep3 (**CVIIIi**), Pep4 (**CVIIIj**), Pep5 (**CVIIIk**) or Pep6 (**CVIIIl**).

Example 9

Octacationic DNA Binding Templates with Dual Ligands

With at least 8 DNA binding templates and at least 12 receptor ligands, there are many possible combinations which can be used. Representative examples include polyamine templates with either a cleavable or non-cleavable spacer joining the templates and oligopeptide templates with either a cleavable or non-cleavable spacer joining the template.

A schematic flow chart for the synthesis of these compounds is shown in Figure 12. The chemical pathway of synthesis is shown below.

For polyamine templates:

Examples 7-10 are all solid phase peptide synthesis. Final coupling are the same for each peptide as described for the **A**, **A'**, **B**, **B'**, **G**, **G'** ligands. Reaction conditions for **D**, **E**, **F** and the Fab' are comparable.

LXXXI + OH^-

↓

t-BOC-HN-$(CH_2)_3$-N(t-BOC)-CH_2-CH(OCH_2-COOH)-$(CH_2)_2$-N(t-BOC)-$(CH_2)_3$-NH_2 (**CIX**)

↓ + FMOC-Cl

t-BOC-HN-$(CH_2)_3$-N(t-BOC)-CH_2-CH(OCH_2-COOH)-$(CH_2)_2$-N(t-BOC)-$(CH_2)_3$-NH-FMOC (**CX**)

In reactions similar to those in Example 7 the following products are found. One skilled in the art will recognize that the starting material and resulting products are different but the reaction is the same.

CX + $H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-R$

where R is **A**, **B** or **G.**

↓

t-BOC t-BOC

t-BOC-HN-$(CH_2)_3$-N-CH_2-CH-$(CH_2)_2$-N-$(CH_2)_3$-NH-FMOC

$OCH_2-COHN-CH_2CH_2-S-S-CH_2CH_2-NH-R$

where R is **A** (**CXIa**), **B** (**CXIb**) or **G** (**CXIc**).

↓ + OH^-

t-BOC t-BOC

t-BOC-HN-$(CH_2)_3$-N-CH_2-CH-$(CH_2)_2$-N-$(CH_2)_3$-NH_2

$OCH_2-COHN-CH_2CH_2-S-S-CH_2CH_2-NH-R$

where R is A (**CXIIa**), B (**CXIIb**) or G (**CXIIc**).

Dissolve 2 mmol **CX** in 2 mL dry DMF, add 4.0 mmol 1-ethyl-3-[3 (dimethylamino)propyl) carbondiimide and stir 2 hr, then add 2.1 mmol N-hydroxysuccinimide and continue stirring for another 6 hr at room temperature, then combine with 2 mmol S(2-aminoethyl) S' (2-pyridyl)-dithiol in 2 mL dry DMF. After stirring overnight at room temperature, remove the solvent *in vacuo*, separate the product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate, 0 to 50% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

↓

t-BOC t-BOC

t-BOC-HN-$(CH_2)_3$-N-CH_2-CH-$(CH_2)_2$-N-$(CH_2)_3$-NH-FMOC

$OCH_2-COHN-CH_2CH_2-S-S-C_5H_4N$ (**CXIII**)

Dissolve 2 mmol **CVIII** in 10 mL ethanol containing 20 mmol piperidine. After overnight at room temperature, the solution is transferred to a separatory funnel, to which 50 mmol of HCl, 5 mL of water and 25 mL of benzene is added. After extraction with 3

additional portions of benzene, the combined organic phase is taken to dryness *in vacuo*. Separate the desired product by solid phase extraction on phenyl-silica and elution with a linear gradient of ethyl acetate to 100% in hexane. Pool the appropriate fractions and evaporate the solvent to obtain the product as an amorphous solid.

$$\text{t-BOC-HN-}(CH_2)_3\text{-}\underset{}{\overset{\text{t-BOC}}{\overset{|}{N}}}\text{-}CH_2\text{-}\underset{\underset{OCH_2\text{-COHN-}CH_2CH_2\text{-S-S-}C_5H_4N}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{\text{t-BOC}}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}NH_2 \qquad \textbf{(CXIV)}$$

Using the same procedures for **CXIII**, except substituting S-*t*-BOC-mercaptoethylamine for $H_2N\text{-}CH_2CH_2\text{-SS-}C_5H_4N$.

$$\textbf{CX} \quad + \quad H_2N\text{-}CH_2CH_2\text{-S-}t\text{-BOC}$$

$$\downarrow$$

$$\text{t-BOC-HN-}(CH_2)_3\text{-}\overset{\text{t-BOC}}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{\underset{OCH_2\text{-COHN-}CH_2CH_2\text{-S-}t\text{-BOC}}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{\text{t-BOC}}{\overset{|}{N}}\text{-}(CH_2)_3\text{-NH-FMOC} \qquad \textbf{(CXV)}$$

$$\downarrow \quad + \quad OH^-$$

$$\text{t-BOC-HN-}(CH_2)_3\text{-}\overset{\text{t-BOC}}{\overset{|}{N}}\text{-}CH_2\text{-}\underset{\underset{OCH_2\text{-COHN-}CH_2CH_2\text{-S-}t\text{-BOC}}{|}}{CH}\text{-}(CH_2)_2\text{-}\overset{\text{t-BOC}}{\overset{|}{N}}\text{-}(CH_2)_3\text{-}NH_2 \qquad \textbf{(CXVI)}$$

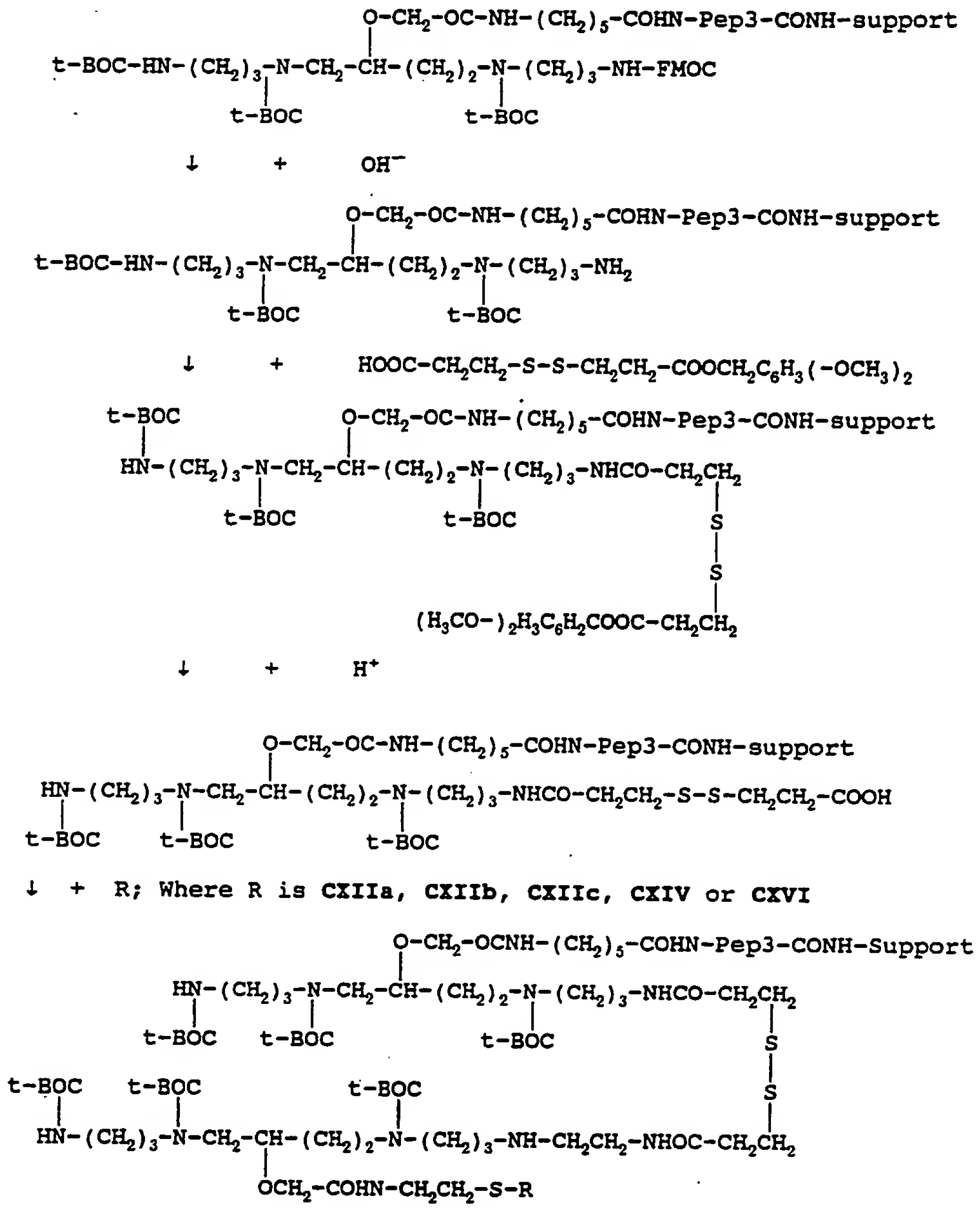
The nuclear localization sequences are added. Standard continuous-flow solid phase synthetic methodologies are used to couple the commercially available 5-(N-*t*-BOC) aminohexanoic acid to the protected peptide on the solid support and the subsequent reaction to give **CXVIIa-c**, **CXVIII** and **CXIX**, as the final products after deprotection and release from the support and chromatographic isolation.

$$H_2N\text{-Pep3-CONH-support} \quad + \quad \text{t-BOC-NH-}(CH_2)_5\text{-COOH}$$

$$\downarrow$$

$$NH_2\text{-}(CH_2)_5\text{-CONH-Pep3-CONH-support}$$

$$\downarrow \quad + \quad \textbf{CX}$$



Where R is $S\text{-}CH_2CH_2\text{-}NH\text{-}A$, $S\text{-}CH_2CH_2\text{-}NHCO\text{-}B$, $S\text{-}CH_2CH_2\text{-}NHCO\text{-}L$, $S\text{-}C_5H_4N$ or S-t-BOC

↓

$$
\begin{array}{l}
\qquad\qquad\qquad\qquad\quad \text{O-CH}_2\text{-OC-NH-(CH}_2)_5\text{-COHN-Pep3-CONH}_2 \\
\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\quad | \\
\qquad\qquad\qquad\qquad\quad \text{OCH}_2\text{-COHN-CH}_2\text{CH}_2\text{-S-R}
\end{array}
$$

Where R is S-CH_2CH_2-NH-**A** (**CXVIIa**), S-CH_2CH_2-NHCO-**B** (**CXVIIb**), S-CH_2CH_2-NHCO-**G** (**CXVIIc**), S-C_5H_4N (**CXVIII**) or SH. (**CXIX**).

To a stirred solution of 10 mg of Fab'-SH in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 **CXVIII** in PBS, pH 7.4, dropwise. After 60 min, dialyze against 3 changes of 0.5 L PBS pH 7.4, at 4°, each for 2 hr. Alternatively to a stirred solution of HS-CH_2CH_2-CONH-Pep2-COOH, prepared by standard solid phase peptide methodology, 10 mg in 5 mL PBS, pH 7.4, at 4°, add 0.3 mL of 10 mM **CXVIII** in PBS, pH 7.4, dropwise. After 60 min,

CXVIII + HR Where R is S-Fab' (**CXXg**)
or S-CH_2CH_2-CONH-Pep2-COOH (**CXXh**).

↓

$$
\begin{array}{l}
\qquad\qquad\qquad\qquad\quad \text{O-CH}_2\text{-OC-NH-(CH}_2)_5\text{-COHN-Pep3-CONH}_2 \\
\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\quad | \\
\qquad\qquad\qquad\qquad\quad \text{OCH}_2\text{-COHN-CH}_2\text{CH}_2\text{-S-R}
\end{array}
$$

Where R is S-Fab' (**CXXg**) or S-CH_2CH_2-CONH-Pep2-COOH (**CXXh**).

Using similar reaction conditions as for preparing **CIIId-f**

CXIX + C_5H_4N-S-S-CH_2CH_2-CONH-R
where R is **D**, **E** or **F**.

↓

$$
\begin{array}{l}
\qquad\qquad\qquad\qquad\quad \text{O-CH}_2\text{-OC-NH-(CH}_2)_5\text{-COHN-Pep3-CONH}_2 \\
\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\
\text{H}_2\text{N-(CH}_2)_3\text{-NH-CH}_2\text{-CH-(CH}_2)_2\text{-NH-(CH}_2)_3\text{-NHCO-CH}_2\text{CH}_2\text{-S} \\
\qquad\qquad\qquad\qquad\quad | \\
\qquad\qquad\qquad\qquad\quad \text{OCH}_2\text{-COHN-CH}_2\text{CH}_2\text{-S-R}
\end{array}
$$

where R is **D** (**CXXId**), **E** (**CXXIe**) or **F** (**CXXIf**).

Substitution of succinic anhydride for HOOC-CH_2CH_2-S-S-CH_2CH_2-$COOH_2$-C_6H_3(-OCH_3)$_2$ at the fourth stage of synthesis gives a noncleavable intermediate which is further modified according to the reaction

sequences for **CXVIIa**, **CXVIIb**, **CXXg**, **CXXh**. **CXXId**, **CXXIe** and **CXXIf** to give the following products for gene delivery.

Standard continuous-flow solid phase synthetic methodologies are used to couple succinic anhydride to the protected peptide on the solid support and the subsequent reaction to give **CXXIIa-c**, **and CXXVh**, as the final products after deprotection and release from the support and chromatographic isolation. The intermediates corresponding to **CXVIII** and **CXIX**, are deprotected, released from the support, chromatographically purified, and reacted with the appropriate intermediates to give **CXXVg**, **CXXVId-f**, as described for the **CXX** and **CXXI** series.

$$
\begin{array}{l}
\qquad\qquad\qquad\qquad\quad O-CH_2-OC-NH-(CH_2)_5-COHN-Pep3-CONH_2\\
\qquad\qquad\qquad\qquad\quad |\\
H_2N-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NHCO-CH_2\\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |\\
H_2N-(CH_2)_3-NH-CH_2-CH-(CH_2)_2-NH-(CH_2)_3-NHCO-CH_2\\
\qquad\qquad\qquad\qquad\quad |\\
\qquad\qquad\qquad\qquad\quad OCH_2-COHN-CH_2CH_2-S-R
\end{array}
$$

where R is S-CH_2CH_2-NH-A (**CXXIIa**), S-CH_2CH_2-NHCO-B (**CXXIIb**), S-CH_2CH_2-NHCO-G (**CXXIIc**), S-Fab′ (**CXXVg**), S-CH_2CH_2-CONH-Pep1-CONH2 (**CXXVh**), S-CH_2CH_2CO-D (**CXXVId**), S-CH_2CH_2-CO-E (**CXXVIe**) or S-CH_2CH_2-CONH-F (**CXXVIf**)

Example 10

Further Octacationic DNA Binding Templates with Dual Ligands

A schematic flow chart for the synthesis of these compounds is shown in Figure 13. The chemical pathway of synthesis is shown below.

Examples 7-10 are all solid phase peptide synthesis. Final coupling are the same for each peptide as described for the **A**, **A′**, **B**, **B′**, **G**, **G′** ligands. Reaction conditions for **D**, **E**, **F** and the Fab′ are comparable.

For oligopeptide template

$$
\begin{array}{l}
\qquad\qquad\qquad\quad t-BOC-HN-tyr-lys-Pep11-CO-support\\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |\\
H_2NOC-Pep3-HNOC-(CH_2)_2-OCHN-(CH_2)_4\\
\qquad \downarrow \qquad H^+\\
\qquad\qquad\qquad\qquad\quad H_2N-tyr-lys-Pep11-CO-support\\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |\\
H_2NOC-Pep3-HNOC-(CH_2)_2-OCHN-(CH_2)_4\\
\qquad \downarrow \qquad + \qquad FMOC-NH-CH_2CH_2-S-S-CH_2CH_2-COOH
\end{array}
$$

$FMOC\text{-}NH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2CONH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}CO\text{-}support$

$H_2NOC\text{-}Pep3\text{-}HNOC\text{-}(CH_2)_2\text{-}OCHN\text{-}(CH_2)_4$

$\downarrow \quad OH^-$

$NH_2\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}CONH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}CO\text{-}support$

$H_2NOC\text{-}Pep3\text{-}HNOC\text{-}(CH_2)_2\text{-}OCHN\text{-}(CH_2)_4$

Further extension of template yields:

$(CH_2)_4\text{-}\epsilon NH_2$

$S\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}Pep11\text{-}lys\text{-}tyr\text{-}NH\text{-}t\text{-}BOC$

$S\text{-}CH_2CH_2\text{-}CONH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}CO\text{-}support$

$CH_2)_4NHCO\text{-}(CH_2)_2\text{-}CONH\text{-}Pep3\text{-}CONH_2$

After derivatization of the ϵ-N-lys with succinic anhydride the ligands (X) are coupled to the resin bound protective peptide.

X where X is $H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}C_5H_4N$,
$H_2N\text{-}CH_2CH_2\text{-}S\text{-}t\text{-}BOC$ or
$H_2N\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}NH\text{-}R$ and
where R is **A**, **B** or **G**.

Although not necessary, it is sometimes desirable to derivatize the amino-terminal tyr with a 2-methoxy-6-chloracridinyl moiety. If not, then deprotection and release from the resin yields:

$(CH_2)_4\text{-}\epsilon NH\text{-}CO\text{-}CH_2CH_2\text{-}CONH\text{-}CH_2CH_2\text{-}S\text{-}R$

$S\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}Pep11\text{-}lys\text{-}tyr\text{-}NH_2$

$S\text{-}CH_2CH_2\text{-}CONH\text{-}tyr\text{-}lys\text{-}Pep11\text{-}COOH$

$(CH_2)_4NHCO\text{-}(CH_2)_2\text{-}CONH\text{-}Pep3\text{-}CONH_2$

Where R is $S\text{-}CH_2CH_2\text{-}NHCO\text{-}$**A** (**CXXVIIa**), $S\text{-}CH_2CH_2\text{-}NHCO\text{-}$**B** (**CXXVIIb**), $S\text{-}CH_2CH_2\text{-}NHCO\text{-}$**G** (**CXXVIIc**), $S\text{-}C_5H_4N$ (**CXXIX**) or SH (**CXXX**).

CXXX + $C_5H_4N\text{-}S\text{-}S\text{-}CH_2CH_2\text{-}CONH\text{-}R$; where R is **D**, **E** or **F**

$\downarrow$

```
                                              R-HNOC-CH2CH2-S
                                                            |
                        (CH2)4-εNH-CO-CH2CH2-CONH-CH2CH2-S
                         |
S-CH2-CH2-NH-CO-Pep11-lys-tyr-NH2
|
S-CH2-CH2CONH-tyr-lys-Pep11-COOH
                   |
                  (CH2)4NHCO-(CH2)2-CONH-Pep3-CONH2
```

Where R is D (**CXXXId**), E (**CXXXIe**) or F (**CXXXIf**)

After derivatization of the ε-N-succinyl-lys with H_2N-CH_2CH_2-S-S-CH_2CH_2-NH-t-BOC and deblocking the ligand Pep3 is synthesized on the resin using standard solid phase techniques. Deblocking and cleavage from the resin yields:

```
                                       HOOC-Pep2-HNOC-CH2CH2-S
                                                            |
                        (CH2)4εNH-CO-CH2CH2-CONH-CH2CH2-S
                         |
S-CH2-CH2-NH-CO-Pep11-lys-tyr-NH2
|                                                      (CXXXIIh)
S-CH2-CH2-CONH-tyr-lys-Pep11-COOH
                    |
                   (CH2)4NHCO-(CH2)2-CONH-Pep3-CONH2
```

```
                CXXXIX        +     Fab'-SH

                         ↓          Fab'-R-HNOC-CH2CH2-S
                                                     |
                        (CH2)4εNH-CO-CH2CH2-CONH-CH2CH2-S
                         |
S-CH2-CH2-NH-CO-Pep11-lys-tyr-NH2
|                                                      (CXXXIIIg)
S-CH2-CH2-CONH-tyr-lys-Pep11-COOH
                    |
                   (CH2)4NHCO-(CH2)2-CONH-Pep3-CONH2
```

Substitution of t-BOC-NH-$(CH_2)_5$-COOH for FMOC-NH-CH_2CH_2-S-S-CH_2-CH_2-COOH at the second stage of synthesis gives a noncleavable intermediate, which is further modified according to the reaction sequence for the **CXXVII** series, **CXXXg**, **CXXXIIIh** and the **CXXXI** series to give the following products for gene delivery.

```
                                              R-HNOC-CH2CH2-S
                                                            |
                        (CH2)4εNH-CO-CH2CH2-CONH-CH2CH2-S
                         |
CH2-(CH2)4-NH-CO-Pep11-lys-tyr-NH2
|
CONH-tyr-lys-Pep11-COOH
          |
         (CH2)4NHCO-(CH2)2-CONH-Pep3-CONH2
```

where R is S-CH_2CH_2-NH-**A** (**CXXXIVa**), S-CH_2CH_2-NHCO-**B** (**CXXXIVb**), S-CH_2CH_2-NHCO-**L** (**CXXXIVc**), S-Fab' (**CXXXIXg**), S-CH_2CH_2-CONH-Pep1-$CONH_2$ (**CXXXVIIIh**), S-CH_2CH_2.CO-**D** (**CXXXVIId**), S-CH_2CH_2-CO-**E** (**CXXXVIIe**), S-CH_2CH_2-CONH-**F** (**CXXXVIIf**).

Example 11

Oligonucleotides Containing Receptor Ligands

Figure 14 show a double stranded DNA vector with a single stranded DNA attached to a ligand containing both a plasma membrane and nuclear membrane receptors. Two different functions can be shown for this single stranded pyrimidine deoxyoligonucleotide modified at the 3' and 5' terminal nucleotides with a space molecule derivatized with a ligand for either a plasma membrane receptor or nuclear membrane receptor and/or a nucleotide containing a modified base conjugated with a ligand for either a plasma membrane receptor or a nuclear membrane receptor.

The first function is to target double stranded vectors to specific cells and then to the nucleus of the targeted cell for expression of the vector and/or integration of the vector sequences into the host genome. One to ten copies of the double stranded target sequences either individually or clustered will be inserted in non-coding regions of the vector.

The second function is to deliver therapeutic single stranded DNA for treatment of cancer, infectious disease and cardiovascular disease. Targeting the single stranded DNA to specific cells and then to the nucleus of the targeted cell will form a triplex structure that prevents transcription of the specified genes.

In Figure 15A is shown single stranded DNA as a DNA-binding template containing a single receptor ligand. C^m is the 5-methyl cytosine derivative.

In Figure 15B is shown single stranded DNA-binding template in which N-(2-ethylamino)glycine replaces the deoxyribose-phosphate backbone of the nucleic acid polymer.

Derivatives and analogs of Figure 15 are shown in Figures 15C.

In Figure 15D is an example of a ligand containing template in the pyrimidine series. With at least 18 binding templates and at least 12 receptor ligands there are many possible combinations for use. It is obvious to one skilled in the art that two different DNA binding templates could be linked 5'(3') to 3'(5') with a dithio bridge so that the single stranded DNA bearing the plasma membrane ligand would disassociate from the double stranded DNA vector. The oligonucleotides

are made by conventional solid phase synthesis. The 5' and 3' nucleotides contained in an amino group in lieu of the 5' and 3' hydroxyl moieties, respectively, of the terminal nucleotides. The nucleotide T-Y is 5-(N-[N-{N-ligand-5-aminohexanoyl}-4-aminobutanoyl]-3-aminoallyl]-2'-deoxyuridine moiety. The nucleotide A-Y is 8-[N-[N-ligand-5-aminohexanoyl]-8-aminohexylamino]-2'-deoxyadenosine moiety.

The ligand for SV-40 sequences is shown in Figure 16. Therapeutic single stranded DNA for the treatment of cancer infectious disease and cardiovascular disease can be delivered to specific cells and then to the nucleus of the targeted cell where it forms triplex structures that prevent transcription of the specified genes. An initial template will contain two different single stranded DNA templates linked 5'(3') to 3'(5') with a dithio bridge, so that as a result of reduction in the cytoplasm, both the single stranded DNA and the plasma membrane ligand will disassociate from the double stranded DNA vectors as separate molecules. The spacer for the plasma membrane ligand also contains a dithio moiety so that the cellular targeting ligand will be released when the complex is present in the cytoplasm. The oligonucleotides are made by conventional solid phase synthesis. The 5' and 3' nucleotides contain an amino group in lieu of the 5' and 3' hydroxyl moiety, respectively, of the terminal nucleotides. Figure 17 shows an example of this for the C-myc promoter.

In Figure 17 the deoxyoligonucleotide strands are synthesized on an automated DNA synthesizer using a solid-phase cyanoethylphosphoramidate method. Commercially available reagents are used to provide a 3' terminal thiol which is reacted with further reacted A (see Example 1) after deblocking and release of the oligonucleotide from the support. Dissolve 2 mmol of the protected peptide N-succinyl-Pep5-$CONH_2$ released from the peptide support in 2 mL dry DMF, and add 4.0 mmol 1-ethyl-3-[3-(dimethylamino)propyl)carbodiimide. Stir for 2 hr, then add 2.1 mmol N-hydroxysuccinimide. Stir for another 6 hr at room temperature, then couple with the deprotected side chain amino group of 5-(N-[N-{N-ligand-5-aminohexanoyl}-4-aminobutanoyl]-3-aminoallyl)-2'-deoxyuridine moiety on the solid support. The nucleotide is terminated with the commercially available Uni-Link AminoModifier (Clontech). The terminal Fmoc amino protecting group be removed and reacted with 6,9-dichloro-2-methoxyacridine before the substituted oligonucleotide is cleaved from the support.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the

invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned as well as those inherent therein. The DNA transporter systems along with the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

What we claim is:

Claims

1. A DNA transporter system for inserting specific DNA into a cell, comprising:

a plurality of a first DNA binding complex, said complex including a first binding molecule capable of non-covalently binding to DNA, said first binding molecule covalently linked to a surface ligand, said ligand capable of binding to a cell surface receptor;

a plurality of a second DNA binding complex, said complex including a second binding molecule capable of non-covalently binding to DNA, said second binding molecule covalently linked to a nuclear ligand, said nuclear ligand capable of recognizing and transporting the transporter system through a nuclear membrane;

wherein said plurality of first and second DNA binding complexes are capable of simultaneously, non-covalently binding to the specific DNA.

2. The transporter of claim 1 wherein,

the first binding molecule is selected from the group consisting of spermine, spermine derivative, histones, cationic peptides and polylysine; and

the second binding molecule is selected from the group consisting of spermine, spermine derivative, histones, cationic peptides and polylysine.

3. The transporter of claim 2, wherein the first and second binding molecules are the same molecule and are a spermine derivative.

4. The transporter of claim 1, wherein the surface ligand is a molecule which binds to a receptor selected from the group consisting of folate receptor, biotin receptor, lipoic acid receptor, low density lipoprotein receptor, asialoglycoprotein receptor, insulin-like growth factor type II/cation-independent mannose-6-phosphate receptor, calcitonin gene-related peptide receptor, insulin-like growth factor I receptor, nicotinic acetylcholine receptor, hepatocyte growth factor receptor, endothelin receptors or bile acid receptor.

5. The transporter of claim 1, wherein the surface ligand is a molecule which binds to an IgG antigen.

6. The transporter of claim 1, wherein the surface ligand includes a compound selected from the group consisting of Pep1, Pep2, Pep12, Pep13, Pep14, Pep15, Pep16, Pep17, Pep18, Pep19, Pep20, Pep21, Pep22, Pep23, A, B, G, D, E, P, J, M and Fab'

7. The transporter of claim 1, wherein the nuclear ligand is a molecule selected from the group consisting of Pep3, Pep4, Pep5, Pep6, Pep7, Pep8, Pep9 and Pep10.

8. The transporter of claim 1, wherein the surface ligand and nuclear ligand are attached to their respective binding molecule by a spacer.

9. The transporter of claim 8, wherein the spacer is hydrophilic and has from 6-30 carbons.

10. The transporter of claim 9, wherein the spacer has from 6-16 carbons.

11. The transporter of claim 9, wherein the spacer is a repeating ω-amino acid of the structure

$$[NH-(CH_2-CH_2)_n-CO-]_m$$

wherein n = 1-3 and m = 1-20,

12. The transporter of claim 9, wherein the spacer is a disulfide of the structure

$$(CH_2CH_2-S-S-CH_2CH_2-)_n$$

13. The transporter of claim 19, wherein the spacer is an acid sensitive bifunctional molecule of the structure

$$-CO-CH_2-\underset{\displaystyle COOH}{\underset{|}{C}} = CH-NH-CH_2-CH_2-S-$$

14. The transporter of claim 1, further comprising a plurality of a third DNA binding complex, said complex including a third binding molecule capable of non-covalently binding to DNA, said third binding molecule covalently linked to a virus or a lytic peptide;

wherein said plurality of third DNA binding complexes are capable of simultaneously, non-covalently binding to said specific DNA.

15. The transporter of claim 14 wherein,

the third binding molecule is selected from the group consisting of spermine, spermine derivative, histones, cationic polypeptide and polylysine.

16. The transporter of claim 14, wherein the third binding molecule is spermine derivative.

17. The transporter of claim 14, wherein the surface ligand is a molecule which binds to a receptor selected from the group consisting of folate receptor, biotin receptor, lipoic acid receptor, low density lipoprotein receptor and asialoglycoprotein receptor, insulin-like growth factor type II/cation-independent mannose-6-phosphate receptor, calcitonin gene-related peptide receptor, insulin-like growth factor I

receptor, nicotinic acetylcholine receptor, hepatocyte growth factor receptor, endothelin receptors or bile acid receptor.

18. The transporter of claim 1, wherein the surface ligand is a molecule which binds to an IgG antigen.

19. The transporter of claim 1, wherein the surface ligand includes a compound selected from the group consisting of Pep1, Pep2, Pep12, Pep13, Pep14, Pep15, Pep16, Pep17, Pep18, Pep19, Pep20, Pep21, Pep22, Pep23, **A**, **B**, **G**, **D**, **E**, **P**, **J**, **M** and Fab'.

20. The transporter of claim 14, wherein the nuclear ligand is a molecule selected from the group consisting of Pep3, Pep4, Pep5, Pep6, Pep7, Pep8, Pep9 and Pep10.

21. The transporter of claim 14, wherein the virus is selected from the group consisting of adenovirus, parainfluenza virus, herpes virus, retrovirus and hepatitis virus.

22. The transporter of claim 14, wherein the lytic peptide is selected from the group consisting of Pep24, Pep25 and Pep26.

23. The transporter of claim 14, wherein the virus is adenovirus.

24. The transporter of claim 14, wherein the virus ligand is attached to the binding molecule by a spacer.

25. The transporter of claim 24, wherein the spacer is hydrophilic and has from 6-30 carbons.

26. The transporter of claim 25, wherein the spacer has from 6-16 carbons.

27. The transporter of claim 25, wherein the spacer is a repeating ω-amino acids having a structure of

$$[NH-(CH_2-CH_2)_n-CO-]_m$$

where n = 1-3 and m = 1-20,

28. The transporter of claim 25, wherein the spacer is a disulfide having the structure of

$$(CH_2CH_2-S-S-CH_2-CH_2-).$$

29. The transporter of claim 14, wherein the spacer is an acid sensitive bifunctional molelcule of the structure

$$-CO-CH_2-\underset{\displaystyle COOH}{\underset{|}{C}} = CH-NH-CH_2-CH_2-S-$$

30. The transporter of claim 14, wherein the lytic peptide is adenovirus.

31. The transporter of claim 14, wherein the lytic peptide ligand is attached to the binding molecule by a spacer.

32. The transporter of claim 24, wherein the spacer is hydrophilic and has from 6-30 carbons.

33. The transporter of claim 25, wherein the spacer has from 6-16 carbons.

34. The transporter of claim 25, wherein the spacer is a repeating ω-amino acids having a structure of

$$[NH-(CH_2-CH_2)_n-CO-]_m$$

where n = 1-3 and m = 1-20,

35. The transporter of claim 25, wherein the spacer is a disulfide having the structure of

$$(CH_2CH_2-S-S-CH_2-CH_2-).$$

36. The transporter of claim 14, wherein the spacer is an acid sensitive bifunctional molelcule of the structure

$$-CO-CH_2-\underset{\displaystyle COOH}{\underset{|}{C}} = CH-NH-CH_2-CH_2-S-$$

37. A DNA transporter for inserting specific DNA into a cell, comprising

a plurality of a first DNA binding complex, said complex including a first binding molecule capable of non-covalently binding to DNA, said first binding molecule attached to a first spacer, said first spacer also attached to a surface ligand, said ligand capable of binding to a cell surface receptor;

a plurality of a second DNA binding complex, said complex including a second binding molecule capable of non-covalently binding to DNA, said second binding molecule attached to a second spacer, said second spacer also attached to a nuclear ligand capable of recognizing and transporting the transporter system through a nuclear membrane;

a plurality of a third DNA binding complex, said complex including a third binding molecule capable of non-covalently binding to DNA, said third binding molecule attached to a third spacer, said third spacer also attached to a virus or lytic peptide;

wherein said plurality of first, second and third DNA binding complexes are capable of simultaneously, non-covalently binding to the specific DNA.

38. The DNA transporter of claim 37, wherein

the first, second and third binding molecules are a spermine derivative;

the surface ligand is a molecule which binds to the asialoglycoprotein;

the nuclear ligand is selected from the group consisting of Pap3, Pep4, Pep5, Pep6, Pep7, Pep8, Pep9 and Pep10;

the virus is the adenovirus;

the first, second and third spacer is hydrophilic and contains 6-16 carbons.

39. A DNA transporter system for inserting specific DNA into a cell, comprising:

a plurality of a common DNA binding complex, each of said complexes includes a binding molecule capable of non-covalently binding to DNA, said binding molecule attached to both a surface ligand capable of binding to a cell surface receptor and a nuclear receptor capable of recognizing and transporting the transporter system though a nuclear membrane.

40. The transporter of claim 39, further comprising a spacer linking the surface ligand and nuclear ligand to the binding molecule.

41. The transporter of claim 39, further comprising a virus attached to said binding molecule.

42. The transporter of claim 41, further comprising a spacer linking the virus to the binding molecule.

43. A method of introducing DNA into a cell comprising the step of contacting the cell with the DNA transporter of claim 1.

44. A method for *in vivo* targeting of the insertion of DNA into a cell comprising the step of contacting the cell with the DNA transporter of claim 1, wherein the surface receptor is specific to the cell which is targeted.

45. A method for prevention or treatment of disease comprising the step of contacting a cell within an organism with a therapeutic dose of the DNA transporter of claim 1, wherein the specific DNA includes a molecule to treat the disease.

46. The method of claim 45, wherein the organism is a human.

47. The method of claim 45, wherein the organism is an animal.

48. A method for modifying animals comprising the step of contacting a cell within the animal with a DNA transporter of claim 1, wherein the specific DNA includes the sequence to modify the animal.

49. A compound selected from the group consisting of **Xa**, **Xb**, **Xc**, **XIa**, **XIb**, **XIc**, **XXXIXa**, **XXXIXb**, **XXXIXc**, **LIXa**, **LIXb**, **LIXc**, **LXXXVIIa**, **LXXXVIIb** and **LXXXVIIc**.

50. A compound selected from the group consisting of **XIIId**, **XIIIe**, **XLIId**, **XLIIe**, **LXIId**, **LXIIe**, **XCId** and **XCIe**.

51. A compound selected from the group consisting of **XIIIf**, **XLIIf**, **LXIIf** and **XCIf**.

52. A compound selected from the group consisting of **XVIIg**, **XLVIg**, **LXVIg** and **XCVg**.

53. A compound selected from the group consisting of **XXIXg**, **LXXVIIIg** and **CVIg**.

54. A compound selected from the group consisting of **XXVIIh**, **LXXVIh** and **CIVh**.

55. A compound selected from the group consisting of **XXIIi**, **XXIIj**, **XXIIk**, **XXIIl**, **LIi**, **LIj**, **LIk**, **LIl**, **LXXIi**, **LXXIj**, **LXXIk**, **LXXIl**, **XCXi**, **XCXj**, **XCXk** and **XCXl**.

56. A compound selected from the group consisting of **XIXh**, **XLVIIIh**, **LXVIIh** and **XCVIIh**.

57. A compound selected from the group consisting of **XXXi**, **XXXj**, **XXXk**, **XXXl**, **XXXIi**, **XXXIj**, **XXXIk**, **XXXIl**, **LXXIXi**, **LXXIXj**, **LXXIXk**, **LXXIXl**, **LXXXi**, **LXXXj**, **LXXXk**, **LXXXl**, **CVIIi**, **CVIIj**, **CVIIk**, **CVIIl**, **CVIIIi**, **CVIIIj**, **CVIIIk** and **CVIIIl**.

58. A compound selected from the group consisting of **A**, **A'** , **B**, **B'** , **G**, **G'** , and **M**.

59. A compound of the structure of **F**.

60. A compound of the structure **E**.

61. A compound of the structure **D**.

62. A compound selected from the group consisting of **CXLIi**, **CXLIj**, **CXLIk**, **CXLIl**, **CXLIIi**, **CXLIIj**, **CXLIIk** and **CXLIIl**.

63. A compound of the structure **CXLIII**.

64. A compound selected from the group consisting of **XVIII**, **XLVII**, **LXVII**, **XCVI** and **CXIII**.

65. A compound of the structure **CXIX**.

66. A compound selected from the group consisting of **CXLak**, **CXLbk** and **CXLck**.

67. A compound selected from the group consisting of **CXLdk** and **CXLek**.

68. A compound of the structure **CXLgk**.

69. A compound of the structure **CXLhk**.

70. A compound of the structure **CXLfk**.

71. A compound selected from the group consisting of **CXVIIa**, **CXVIIb**, **CXVIIc**, **CXXIIa**, **CXXIIb** and **CXXIIc**.

72. A compound of the structure **CXXg** or **CXXVg**.

73. A compound of the structure **CXXh** or **CXXVh**.

74. A compound selected from the group consisting of **CXXId**, **CXXIe**, **CXXVId** and **CXXVIe**.

75. A compound of the structure **CXXIf** or **CXXVIf**.

76. A compound selected from the group consisting of **XXVId**, **XXVIe**, **LXXVd**, **LXXVe**, **CIIId** and **CIIIe**.

77. A compound selected from the group consisting of **XXVIf**, **LXXVf** and **CIIIf**.

78. A compound selected from the group consisting of **XVI**, **LXV**, **XCIV** and **CXVI**.

79. A compound selected from the group consisting of **IV**, **XXXIII**, **XI**, **XII**, **XL**, **XLI**, **LX**, **LXI**, **LXXXVIII** and **CX**.

80. A compound selected from the group consisting of **XV**, **LXIV**, **XCIII**, **XLV** and **CXIV**.

81. A compound selected from the group consisting of **XXIIIa**, **XXIIIb**, **XXIIIc**, **LXXIIa**, **LXXIIb**, **LXXIIc**, **CIa**, **CIb** and **CIc**.

82. A compound selected from the group consisting of **XXIV**, **XXV**, **LXXIII**, **LXXIV** and **CII**.

83. A compound selected from the group consisting of **VII** and **XXXVI**.

84. A compound selected from the group consisting of **XXVIII**, **LXXVII** and **CV**.

85. A compound selected from the group consisting of **LIV**, **LVI**, **LXXXII**, **LXXXIV** and **CX**.

86. A compound selected from the group consisting of **XXI**, **L**, **LXX** and **XCIX**.

87. A compound selected from the group consisting of **CXXVIIa**, **CXXVIIb**, **CXXVIIc**, **CXXXIVa**, **CXXXIVb** and **CXXXIVc**.

88. A compound selected from the group consisting of **CXXXId**, **CXXXIe**, **CXXXVIId** and **CXXXVIIe**.

89. A compound selected from the group consisting of **CXXXIf** and **CXXXVIIf**.

90. A compound selected from the group consisting of **CXXXIXg** and **CXXXIIIg**.

91. A compound selected from the group consisting of **CXXXIIh** and **CXXXVIIIh**.

92. A compound of the structure **CXXX**.

93. A compound of the structure **CXXIX**.

94. The transporter of claim 1, wherein the plurality of first DNA binding complex are selected from the group consisting of **Xa**, **Xb**, **Xc**, **XIa**, **XIb**, **XIc**, **XXXIXa**, **XXXIXb**, **XXXIXc**, **LIXa**, **LIXb**, **LIXc**, **LXXXVIIa**, **LXXXVIIb**, **LXXXVIIc**, **XIIId**, **XIIIe**, **XLIId**, **XLIIe**, **LXIId**, **LXIIe**, **XCId**, **XCIe**, **XVIIg**, **XLVIg**, **LXVIg**, **XCVg**, **XXIXg**, **LXXVIIIg**, **CVIg**, **XXVIIh**, **LXXVIh**, **CIVh**, **XIXh**, **XLVIIIh**, **LXVIIh**, **XCVIIh**, **CXLak**, **CXLbk**, **CXLck**, **CXLdk**, **CXLek**, **CXLgk**, **CXLhk**, **CXLfk**, **XXVId**, **XXVIe**, **LXXVd**, **LXXVe**, **CIIId**,

CIIIe, XXIIIa, XXIIIb, XXIIIc, LXXIIa, LXXIIb, LXXIIc, CIa, CIb, CIc and combinations thereof.

95. The transporter of claim 1, wherein the plurality of second DNA binding complex are selected from the group consisting of XXIIi, XXIIj, XXIIk, XXIIl, LIi, LIj, LIk, LIl, LXXIi, LXXIj, LXXIk, LXXIl, XCXi, XCXj, XCXk, XCXl, XXXi, XXXj, XXXk, XXXl, XXXIi, XXXIj, XXXIk, XXXIl, LXXIXi, LXXIXj, LXXIXk, LXXIXl, LXXXi, LXXXj, LXXXk, LXXXl, CVIIi, CVIIj, CVIIk, CVIIl, CVIIIi, CVIIIj, CVIIIk, CVIIIl, CXLIi, CXLIj, CXLIk, CXLIl, CXLIIi, CXLIIj, CXLIIk, CXLIIl and combinations thereof.

96. The transporter of claim 14, wherein the plurality of third DNA binding complex are selected from the group consisting of XIIIf, XLIIf, LXIIf, XCIf, CXXIf, CXXVIf, XXVIf, LXXVf, CIIIf and combinations thereof.

97. The transporter of claim 2, wherein the first an second binding molecules are selected from the group consisting of IV, XXXIII, XI, XII, XL, XLI, LX, LXI, LXXXVIII, CX, XXIV, XXV, LXXIII, LXXIV, CII and combinations thereof.

98. The transporter of claim 39, wherein the plurality of common DNA complex is selected from the group consisting of CXVIIa, CXVIIb, CXVIIc, CXXIIa, CXXIIb, CXXIIc, CXXg, CXXVg, CXXh, CXXVh, CXXId, CXXIe, CXXVId, CXXVIe, CXXVIIa, CXXVIIb, CXXVIIc, CXXXIVa, CXXXIVb, CXXXIVc, CXXXId, CXXXIe, CXXXVIId, CXXXVIIe, CXXXIf, CXXXVIIf, CXXXIXg, CXXXIIIg, CXXXIIh, CXXXVIIIh and combinations thereof.

99. A compound of the structure of P.

100. A compound of the structure of J.

101. A compound of the structure of Q.

102. A compound of the structure shown in Figure 15B.

103. A compound of the structure shown in Figure 15C.

104. A compound of the structure shown in Figure 15D.

105. A compound of the structure shown in Figure 22.

106. A compound of the structure shown in Figure 24.

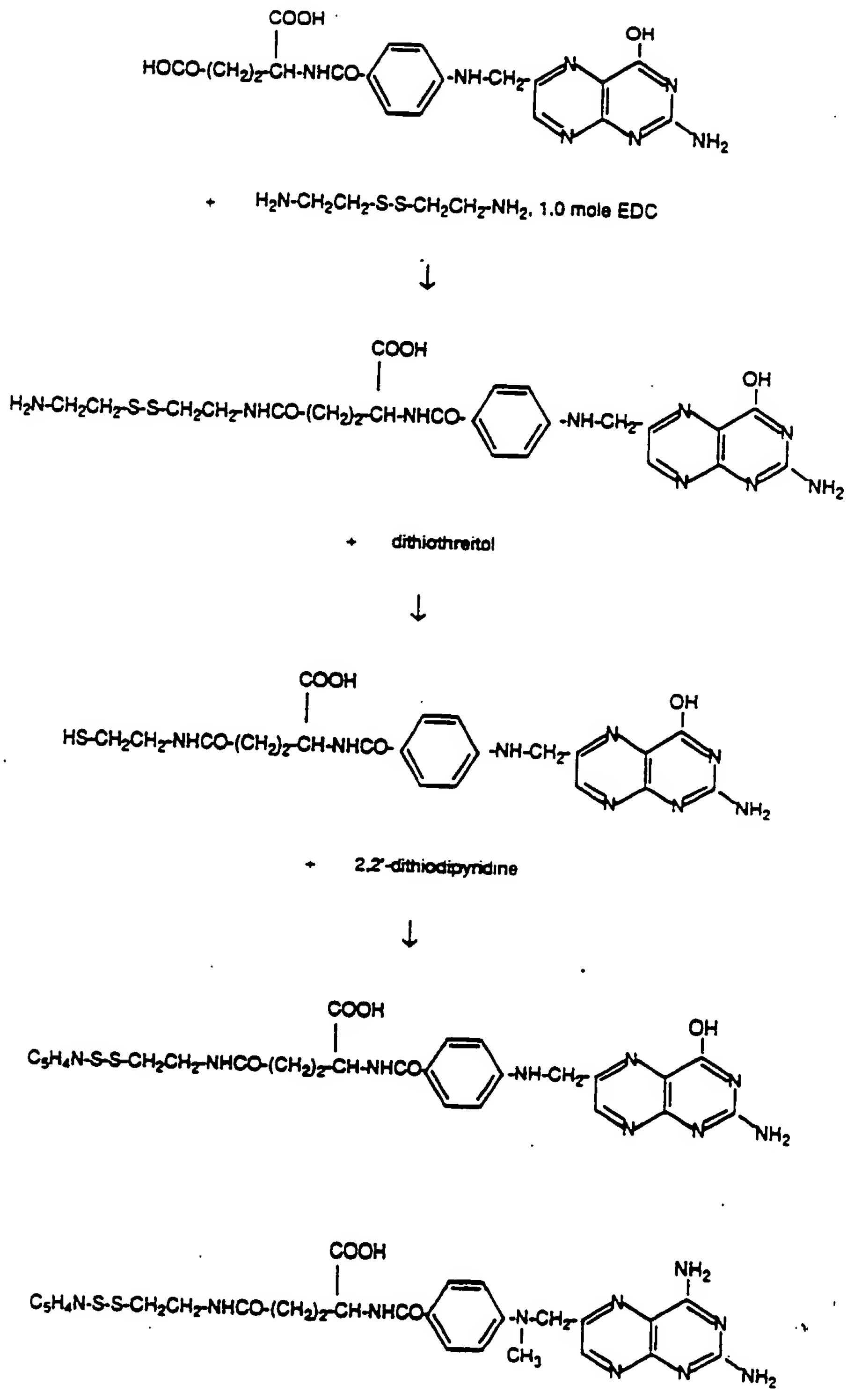
107. A compound of the structure selected from the group consisting of 4, 10, 11, 12, 13, 14, 15 and 16.

108. A compound of the structure selected from the group consisting of 17, 18, 19 and 20.

109. A compound of the structure selected from the group consisting of 6, 21, 22, 23 and 24.

110. A compound of the structure selected from the group consisting of 25, 26 and 27.

111. A compound of the structure selected from the group consisting of 28, 29 and 30.

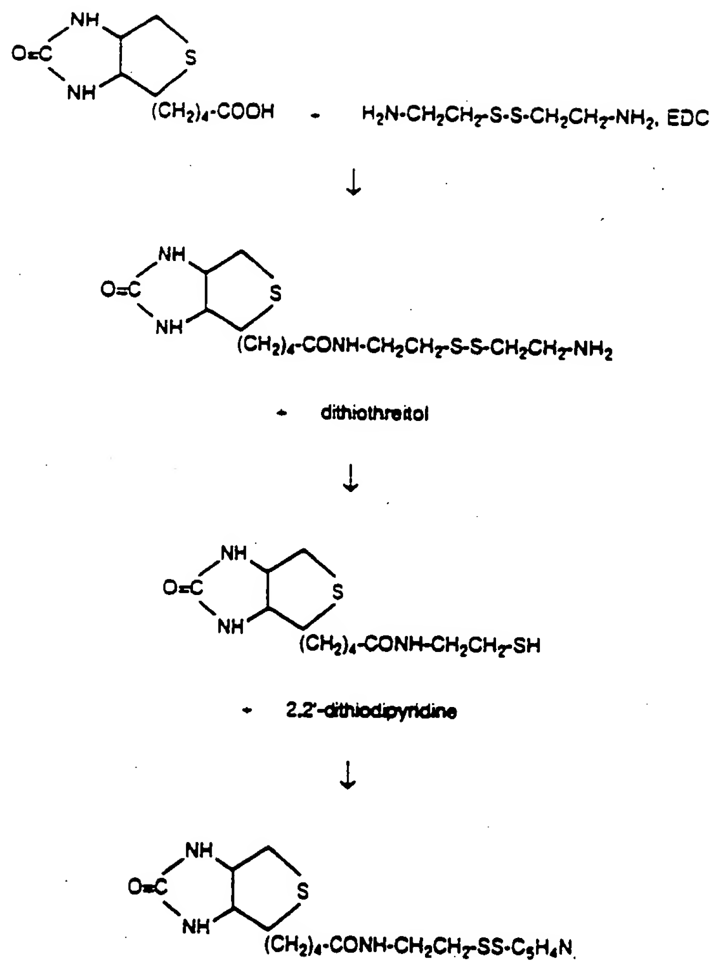

COOH
HOCO-(CH2)2-CH-NHCO-
-NH-CH2-
OH
NH2
+ H2N-CH2CH2-S-S-CH2CH2-NH2, 1.0 mole EDC
H2N-CH2CH2-S-S-CH2CH2-NHCO-(CH2)2-CH-NHCO-
+ dithiothreitol
HS-CH2CH2-NHCO-(CH2)2-CH-NHCO-
+ 2,2'-dithiodipyridine
C5H4N-S-S-CH2CH2-NHCO-(CH2)2-CH-NHCO
-N-CH2-
CH3

(A)

(A')

(M)

Figure 1

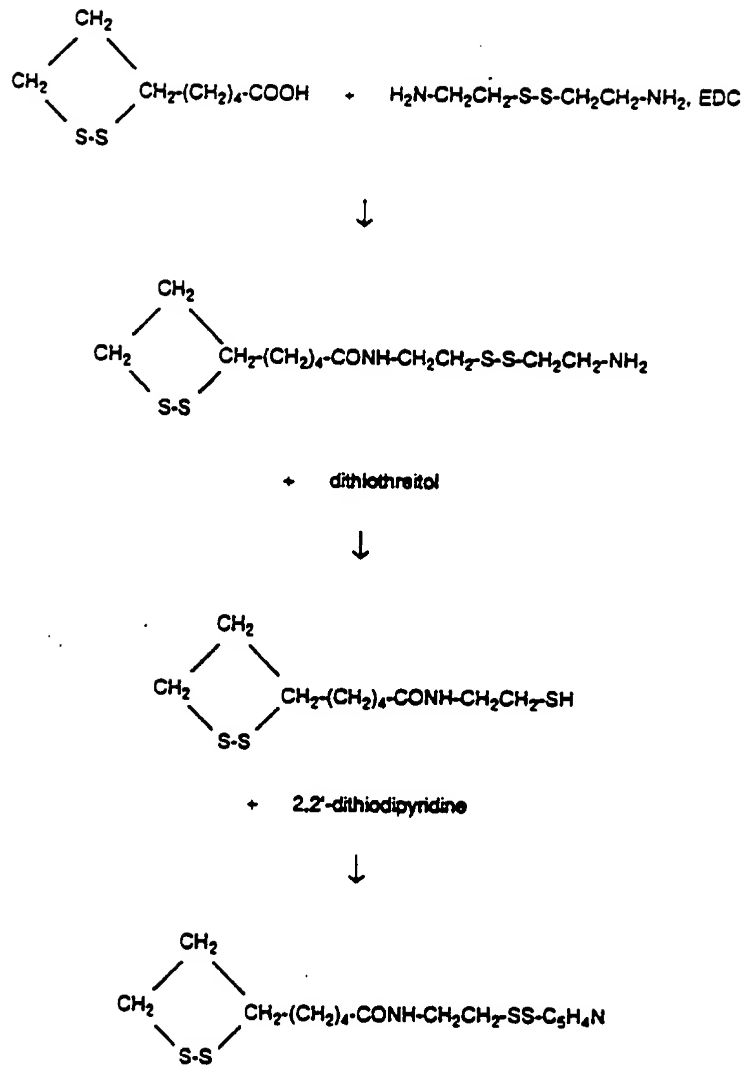


NH
O=C
NH
S
(CH2)4-COOH
+ H2N-CH2CH2-S-S-CH2CH2-NH2, EDC
(CH2)4-CONH-CH2CH2-S-S-CH2CH2-NH2
+ dithiothreitol
(CH2)4-CONH-CH2CH2-SH
+ 2,2'-dithiodipyridine
(CH2)4-CONH-CH2CH2-SS-C5H4N

(B)

(B')

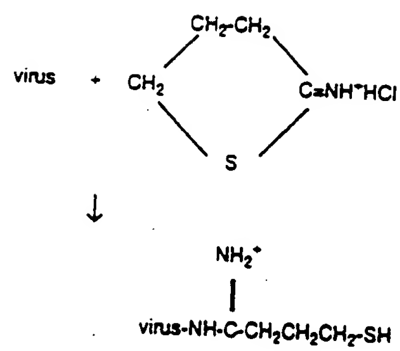
Figure 2


CH2
CH2
CH2-(CH2)4-COOH
+
H2N-CH2CH2-S-S-CH2CH2-NH2, EDC
S-S
CH2-(CH2)4-CONH-CH2CH2-S-S-CH2CH2-NH2
+ dithiothreitol
CH2-(CH2)4-CONH-CH2CH2-SH
+ 2,2'-dithiodipyridine
CH2-(CH2)4-CONH-CH2CH2-SS-C5H4N

(G)

(G')

Figure 3



(F)

Figure 4

VIIIa → Xa
VIIIb → Xb
VIIIc → Xc

I → II → III → IV → V → VI → VII → IX → XI → XII → XIIId, XIIIe, XIIIf

VII ↓ XIV ↓ XV → XVI → XVIIg

VII → XX → XIX → XXIIi, XXIIj, XXIIk, XXIIl

IX → XVIII → XIXh

XII → Xa, Xb, Xc

FIGURE 5

solid phase synthesis:

XXIIIa, XXIIIb, XXIIIc

XXVIIh

XXXi, XXXj, XXXk, XXXl

XXXIi, XXXIj, XXXIk, XXXIl

XXIV → XXV → XXVId, XXVIe, XXVIf

XXVIII → XXIXg

FIGURE 6

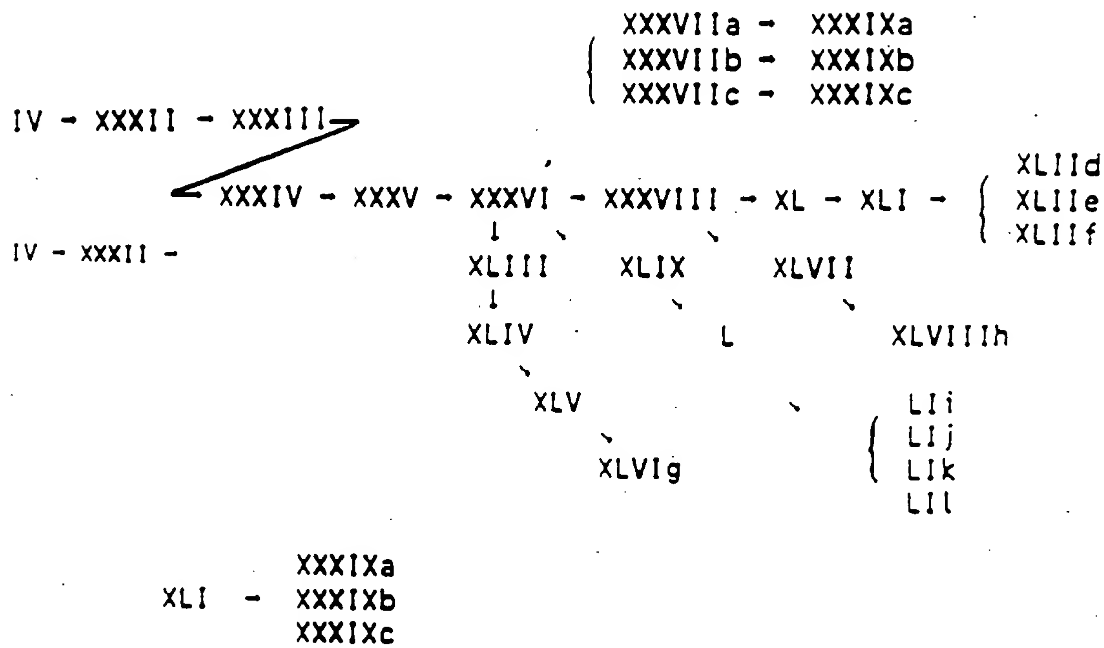


FIGURE 7

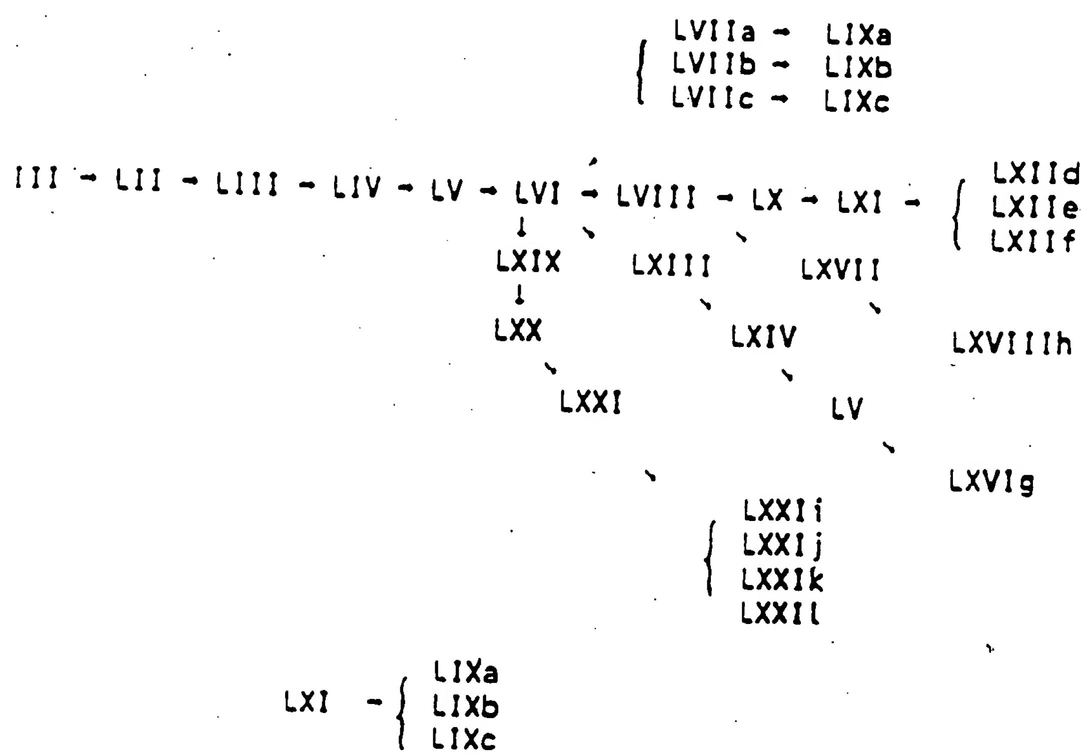


FIGURE 8

solid phase synthesis: LXXIIa LXXIIb LXXIIc LXXVIh LXXIXi LXXIXj LXXIXk LXXIXl LXXXi LXXXj LXXXk LXXXl

LXXIII → LXXIV → { LXXVd, LXXVe, LXXVf }

LXXVII → LXXVIIIg

FIGURE 9

LIV → LXXXI → LXXXIII → LXXXIV → LXXXIX → XC → { XCId, XCIe, XCIf }

LXXXIV → { LXXXVa → LXXXVIIa, LXXXVb → LXXXVIIb, LXXXVc → LXXXVIIc }

LXXXIV → LXXXVI → LXXXVIII → XCVI → XCVIIh

LXXXIV → XCVIII → XCIX → { Ci, Cj, Ck, Cl }

LXXXIV → XCII → XCIII → XCIV → XCVg

LXXXIX → LXXXVIIa, LXXXVIIb, LXXXVIIc

FIGURE 10

solid phase synthesis: CIa CIb CIc CIVh CVIIi CVIIj CVIIk CVIIl CVIIIi CVIIIj CVIIIk CVIIIl

CII → { CIIId, CIIIe, CIIIf }

CV → CVIg

FIGURE 11

CXIa - CXIIa - CXVIIa
CXIb - CXIIb - CXVIIb
CXIc - CXIIc - CXVIIc

LXXXI - CIX → CX - CXIII - CXIV - CXVIIIg
CXVIIIh

CXV

CXVI - CXIX - CXXd
CXXe
CXXf

CXXIII - CXXIVg
CXXIVh

CXXV - CXXVId
CXXVIe
CXXVIf

solid phase synthesis: CXXIIa, CXXIIb, CXXIIc

FIGURE 12

CXXXIX - CXXXIIIg

CXXX - CXXXId
CXXXIe
CXXXIf

solid phase synthesis: CXXVIIa, CXXVIIb, CXXVIIc, CXXXIIh

FIGURE 13

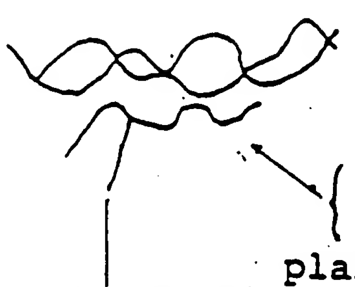


FIGURE 14

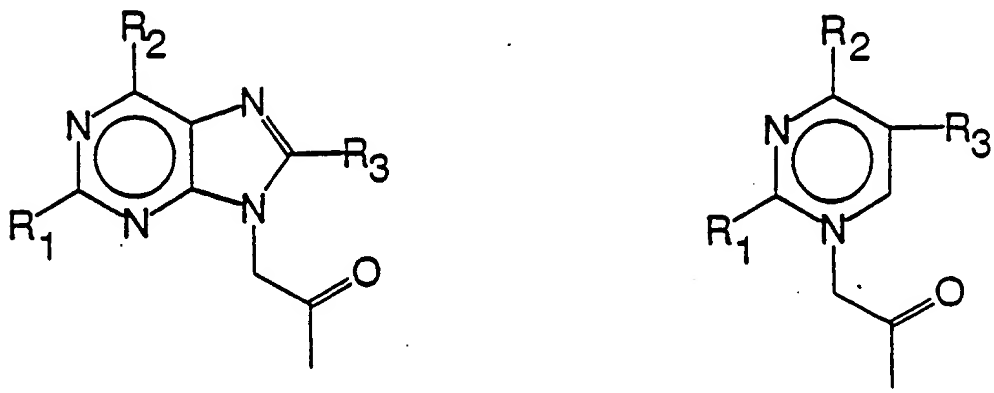
target sequence in double stranded vector	ligand containing templates pyrimidine series	purine series
5'-TCCTAGCAAAGGAGGAGACGAAGAAAAATGA-3' 3'-AGGATCGTTTCCTCCTCTGCTTCTTTTTTAC-5'	5' X-TTT^{m}C^{m}CT^{m}C^{m}CT^{m}CT-Z 3' Y	5' X-AAAGGAGGAGA-Z 3' Y
5'-TCCAAAAAAGAAGAGAAAGGTAG-3' 3'-AGGTTTTTTCTTCTCTTTCCATC-5'	5' X-TTTTTT^{m}CTT^{m}CT^{m}CTTT^{m}C^{m}C-Z 3' Y	5' X-AAAAAAGAAGAGAAAGG-Z 3' Y
5'-AAAGAGAGAGAGAGGGA-3' 3'-GGGAGAGAGAGAGAAAG-5'	5' X-TTT^{m}CT^{m}CT^{m}CT^{m}CT^{m}CT^{m}C^{m}C^{m}CT-Z 3' Y	5' X-AAAGAGAGAGAGAGGGA-Z 3' Y
5'-CTACTCGAGAAAGGAGAGAAAAAGGGGCGGTCCCG-3' 3'-GATGAGCTCTTTCCTCTCTTTTTCCCCGCCAGGGC-5'	5' X-^{m}CT^{m}CTTT^{m}C^{m}CT^{m}CT^{m}CTTTTT^{m}C^{m}C^{m}C^{m}C-Z 3' Y	5' X-GAGAAAGGAGAGAAAAAGGGG-Z 3' Y
5'-CTCTCTAAAAAGGGAGGGGAGGGGAGGGAAAAACTCTCT-3' 3'-GAGAGATTTTTCCCTCCCCTCCCCTCCCTTTTTGAGAGA-5'	5' X-TTTTT^{m}C^{m}C^{m}CT^{m}C^{m}C^{m}C^{m}CT^{m}C^{m}C^{m}C^{m}CT^{m}C^{m}C^{m}CTTTTT-Z 3' Y	5' X-AAAAAGGGAGGGGAGGGGAGGGAAAAA-Z 3' Y

FIGURE 15A

$$R_1\text{-}NH\overset{B}{\overset{|}{C}}OCH_2N\overset{B}{\overset{|}{C}}H_2CH_2NH(\overset{B}{\overset{|}{C}}OCH_2NCH_2CH_2NH)_nCOCH_2NCH_2CH_2NH\text{-}R_2$$

where R_1 and R_2 are spacer molecules; n is the number of repeating units ranging from 20 to 40; and B may be N-carboxymethyl derivatives of thymine, cytosine, adenine, guanine and/or derivatives and analogs thereof

Figure 15B



where R_1, R_2, and R_3 can be H or other substituents

FIGURE 15C

$$\overset{Y}{\overset{|}{N}}H(COCH_2\overset{T}{\overset{|}{N}}CH_2CH_2NH)_3(COCH_2\overset{^mC}{\overset{|}{N}}CH_2CH_2NH)_2COCH_2\overset{T}{\overset{|}{N}}CH_2CH_2NH(COCH_2\overset{^mC}{\overset{|}{N}}CH_2CH_2NH)_2\text{-}$$

$$COCH_2\overset{T}{\overset{|}{N}}CH_2CH_2NHCOCH_2\overset{^mC}{\overset{|}{N}}CH_2CH_2NHCOCH_2\overset{T}{\overset{|}{N}}CH_2CH_2NH\overset{Z}{\overset{|}{C}}O$$

where Y and Z may be spacers terminated in a ligand or an intercalating group.

Figure 15D

Ligand for SV-40 sequences.

5' X-TTT^{m}C^{m}CT^{m}C^{m}CT^{m}CT-Z 3'
with Y attached below the first ^{m}C

where

X = (2-methoxy-6-chloroacridinyl)-NH
Y = 5-$CH_2CH_2CH_2NHCO(CH_2)_3$-$NHCO(CH_2)_5CO$-R_y
Z = $(CH_2)_5$-NH-CH_2CH_2-S-R_z

where R_y

= gly-tyr-ser-thr-pro-gly-arg-lys-lys-arg-$CONH_2$

where R_z

=	-S-CH_2CH_2-NH-γ-amide of the glutamyl moiety of folic acid	(CXLak)
=	-S-CH_2CH_2-NHCO-biotin	(CXLbk)
=	-S-CH_2CH_2-NHCO-lipoic acid	(CXLck)
=	-S-Fab'	(CXLgk)
=	-S-CH_2CH_2-CONH-his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu-gly-$CONH_2$	(CXLhk)

= -S-CH_2
CH_2
CO
NH-CH-CONH-$(CH_2)_5$-COHN-his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu-gly-$CONH_2$ (CXLdk)
|
CH_2-CONH-$(CH_2)_5$-COHN-his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu-gly-$CONH_2$

= -S-CH_2CH_2-CONH-CH-CONH-$(CH_2)_5$-CONH-C-$(CH_2$-O-Lac$)_3$ (CXLek)
|
CH_2-CONH-$(CH_2)_5$-CONH-C-$(CH_2$-O-Lac$)_3$

= -S-CH_2CH_2-CONH-fusion competent adenovirus (CXLfk)

FIGURE 16

DNA binding oligonucleotide		genomic target
5' X-TGGGGAGGGAGGGGAGGGAGGGGAAGG-Z 3' (Y at -148)	-148 … -122	c-myc promoter
5' X-TAGAGGAGGCCGCAGGGGCTGGGCAGGAAGGAGGTGAAT (Y at -145)	-145 … -98	cholesteryl ester transfer protein promoter

1. Ligand for c-myc promoter:

5' X-^{m}CT^{m}CTTT^{m}C^{m}CT^{m}CTTTTT^{m}C^{m}C^{m}C^{m}C-NHCO-CH_2CH_2-SS-CH_2CH_2-CONH-
(with Y_a on the first T)

TGGGGAGGGAGGGGAGGGAGGGGAAGG-Z 3'
(with Y_b on the first T)

where X = (2-methoxy-6-chloroacridinyl)-NH

Y_a = $(CH_2)_3$-NH-CO$(CH_2)_5$-NH-R_2

Y_b = H on the methyl group of the thymidine moiety

Z = CO-$(CH_2)_5$-NH-CH_2CH_2-S-R_2

where R_2 = pro-asp-glu-val-lys-arg-lys-lys-lys-pro-pro-thr-ser-tyr-gly-NH_2 (CXLIi)

= pro-arg-arg-arg-thr-lys-pro-pro-thr-ser-tyr-gly-NH_2 (CXLIj)

= arg-lys-lys-arg-gly-pro-thr-ser-tyr-gly-NH_2 (CXLIk)

= trp-arg-arg-arg-arg-asn-arg-arg-pro-thr-ser-tyr-gly-NH_2 (CXLIl)

= gly-tyr-ser-thr-pro-pro-lys-lys-lys-arg-lys-val-glu-asp-pro-$CONH_2$ (CXLIIi)

= gly-tyr-ser-thr-pro-pro-lys-thr-arg-arg-arg-pro-$CONH_2$ (CXLIIj)

= gly-tyr-ser-thr-pro-gly-arg-lys-lys-arg-$CONH_2$ (CXLIIk)

= gly-tyr-ser-thr-pro-arg-arg-asn-arg-arg-arg-arg-trp-$CONH_2$ (CXLIIl)

where R_2 = -S-CH_2CH_2-NH-λ-amide of the glutamyl moiety of methotrexate (CXLIII)

FIGURE 17

Pep1
his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu-gly

Pep2
his-leu-arg-arg-leu-arg-arg-arg-leu-leu-arg-glu-ala-glu-glu

Pep3
gly-tyr-ser-thr-pro-pro-lys-lys-lys-arg-lys-val-glu-asp-pro

Pep4
gly-tyr-ser-thr-pro-pro-lys-thr-arg-arg-arg-pro

Pep5
gly-tyr-ser-thr-pro-gly-arg-lys-lys-arg

Pep6
gly-tyr-ser-thr-pro-arg-arg-asn-arg-arg-arg-arg-trp

Pep7
pro-asp-glu-val-lys-arg-lys-lys-lys-pro-pro-thr-ser-tyr-gly

Pep8
pro-arg-arg-arg-thr-lys-pro-pro-thr-ser-tyr-gly

Pep9
arg-lys-lys-arg-gly-pro-thr-ser-tyr-gly

Pep10
trp-arg-arg-arg-arg-asn-arg-arg-pro-thr-ser-tyr-gly

Pep11
lys-ala-lys-ala-lys-ala-lys

A = γ-amide of the glutamyl moiety of folic acid (Figure 1)

B = biotin (Figure 2)

G = lipoic acid (Figure 3)

D = NH-CH-CONH-$(CH_2)_5$-COHN-Pep1-$CONH_2$
        |
        CH2-CONH-$(CH_2)_5$-COHN-Pep1-$CONH_2$

E = NH-CH-CONH-$(CH_2)_5$-CONH-C-$(CH_2$-O-Lac$)_3$
        |
        CH_2-CONH-$(CH_2)_5$-CONH-C-$(CH_2$-O-Lac$)_3$

F = fusion competent virus (Figure 4)

Fab' = fragment of IgG

M = γ-amide of the glutaryl moity of methotrexate (Figure 1)

t-Boc = $(CH)_3COCO$ Z = $C_6H_5CH_2OCO$

Figure 18

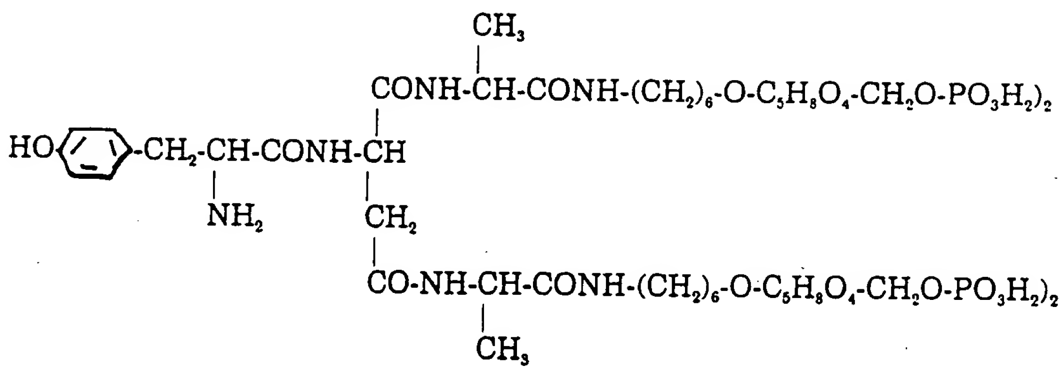


Figure 19

Pep 12

Gln-Ala-Tyr-Arg-Pro-Ser-Glu-Thr-Leu-Cys-Gly-Gly-Glu-Leu-Val-Asp-Thr-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Leu-Phe-Ser-Arg-Pro-Ala-Ser-Arg-Val-Ser-Arg-Arg-Ser-Arg-Gly-Ile-Val-Glu-Glu-Cys-Cys-Phe-Arg-Ser-Cys-Asp-Leu-Ala-Leu-Leu-Glu-Thr-Tyr-Cys-Ala-Thr-Pro-Ala-ε-X-Lys-Ser-Glu

Pep 13

Gln-Ala-Tyr-ε-X-Lys-Pro-Ser-Glu-Thr-Leu-Cys-Gly-Gly-Glu-Leu-Val-Asp-Thr-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Leu-Phe-Ser-Arg-Pro-Ala-Ser-Arg-Val-Ser-Arg-Arg-Ser-Arg-Gly-Ile-Val-Glu-Glu-Cys-Cys-Phe-Arg-Ser-Cys-Asp-Leu-Ala-Leu-Leu-Glu-Thr-Tyr-Cys-Ala-Thr-Pro-Ala-Arg-Ser-Glu

Pep 14

Tyr-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-ε-X-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2

Pep 15

CO-Asp-Thr-Ala-Thr-NH-CH-CO-Tyr-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-

CH_2-$(CH_2)_3$-CH_2

Ser-Gly-Gly-Val-Val-ε-X-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2

Pep 16

Gln-Ala-Tyr-Arg-Pro-Ser-Glu-Thr-Leu-Cys-Gly-Gly-Glu-Leu-Val-Asp-Thr-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Leu-Phe-Ser-Arg-Pro-Ala-Ser-Arg-Val-Ser-Arg-Arg-Ser-Arg-Gly-Ile-Val-Glu-Glu-Cys-Cys-Phe-Arg-Ser-Cys-Asp-Leu-ε-X-Lys-Arg-Leu-Glu-Thr-Tyr-Cys-Ala-Thr-Pro-Ala-Arg-Ser-Glu

Figure 20

Pep 17

Asn-X-Thr-Leu-Cys-Gly-Ala-Glu-Leu-Val-Asp-Ala-Leu-Gln-Phe-Val-Cys-Gly-Asp-Arg-Gly-Phe-Tyr-Phe-Asn-Lys-Pro-Thr-Gly-Tyr-Gly-Ser-Ser-Ser-Arg-Arg-Ala-Pro-Gln-Thr-Gly-Ile-Val-Asp-Glu-Cys-Cys-Phe-Arg-Ser-Cys-Asp-Leu-Arg-Arg-Leu-Glu-Met-Tyr-Cys-Ala-Pro-Leu-Arg-Pro-Ala-Arg-Ser-Ala-Arg-Ser-Val-Arg-Ala-Gln-Arg-His-Thr-Asp

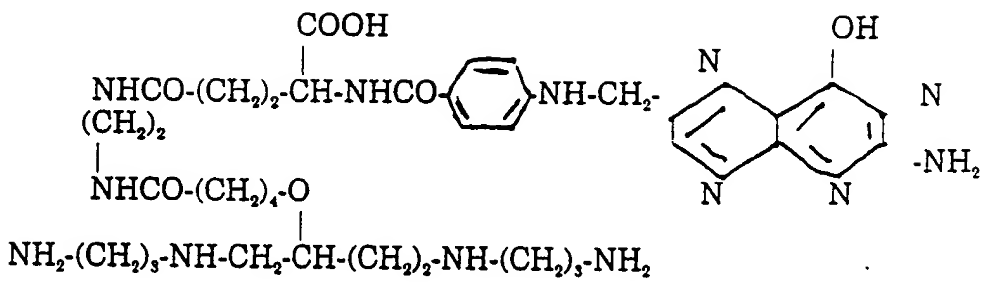
Pep 18

ε-X-Lys-Gly-Leu-Pro-Lys-Glu-Val-Pro-Ala-Val-Leu-Thr-Lys-Gln-Lys-Leu-Lys-Ser-Glu-Leu-Val-Ala-Asn-Gly-Val-Thr-Leu-Pro-Ala-Gly-Glu-Met-Arg-Lys-Asp-Val-Tyr-Val-Glu-Leu-Tyr-Leu-Gln-His-Leu-Thr-Ala-Leu-His

Pep 19

Gly-Leu-Pro-ε-X-Lys-Glu-Val-Pro-Ala-Val-Leu-Thr-Lys-Gln-Lys-Leu-Lys-Ser-Glu-Leu-Val-Ala-Asn-Gly-Val-Thr-Leu-Pro-Ala-Gly-Glu-Met-Arg-Lys-Asp-Val-Tyr-Val-Glu-Leu-Tyr-Leu-Gln-His-Leu-Thr-Ala-Leu-His

Figure 20



Compound Q

Figure 21

$$\mathrm{Y{-}K(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5K{-}CONH\overset{\overset{\mathrm{CONH_2}}{|}}{C}HCH_2{-}SS{-}(CH_2)_3{-}COHN\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5\underset{\mathrm{M6P}}{\underset{|}{K}}(K)_5K{-}COOH}$$

where M6P is the ω-[2-(6-O-phosphoryl-α-D-mannopyranosyl)oxy]alkanoyl moiety on the ε-amino group of lys in the polycation; alkanoyl is $(CH_2)_n$ and n ranges from 3 to 20.

Figure 22

Pep 20

Gln-Arg-Lys-Arg-Arg-Asn-Thr-Ile-His-Glu-Phe-Lys-Lys-Ser-Ala-Lys-Thr-Thr-
Leu-Ile-Lys-Ile-Asp-Pro-Ala-Leu-Lys-Ile-Lys-Thr-Lys-Lys-Val-Asn-Thr-Ala-
Asp-Gln-Cys-Ala-Asn-Arg-Cys-Thr-Arg-Asn-Lys-Gly-Leu-Pro-Phe-Thr-Cys-Lys-
Ala-Phe-Val-Phe-Asp-Lys-Ala-Arg-Lys-Gln-Cys-Leu-Trp-Phe-Pro-Phe-Asn-Ser-
Met-Ser-Ser-Gly-Val-Lys-Lys-Glu-Phe-Gly-His-Glu-Phe-Asp-Leu-Tyr-Glu-Asn-
Lys-Asp-Tyr-Ile-Arg-Asn-Cys-Ile-Ile-Gly-Lys-Gly-Arg-Ser-Tyr-Lys-Gly-Thr-
Val-Ser-Ile-Thr-Lys-Ser-Gly-Ile-Lys-Cys-Gln-Pro-Trp-Ser-Ser-Met-Ile-Pro-
His-Glu-His-Ser-Phe-Leu-Pro-Ser-Ser-Tyr-Arg-Gly-Lys-Asp-Leu-Gln-Glu-Asn-
Tyr-Cys-Arg-Asn-Pro-Arg-Gly-Glu-Glu-Gly-Gly-Pro-Trp-Cys-Phe-Thr-Ser-Asn-
Pro-Glu-Val-Arg-Tyr-Glu-Val-Cys-Asp-Ile-Pro-Gln-Cys-Ser-Glu-Val-Glu-Cys-
Met-Thr-Cys-Asn-Gly-Glu-Ser-Tyr-Arg-Gly-Leu-Met-Asp-His-Thr-Glu-Ser-Gly-
Lys-Ile-Cys-Gln-Arg-Trp-Asp-His-Gln-Thr-Pro-His-Arg-His-Lys-Phe-Leu-Pro-
Glu-Arg-Tyr-Pro-Asp-Lys-Gly-Phe-Asp-Asp-Asn-Tyr-Cys-Arg-Asn-Pro-Asp-Gly-
Gln-Pro-Arg-Pro-Trp-Cys-Tyr-Thr-Leu-Asp-Pro-His-Thr-Arg-Trp-Glu-Tyr-Cys-
Ala-Ile-Lys-Thr-Cys-Ala-Asp-Asn-Thr-Met-Asn-Asp-Thr-Asp-Val-Pro-Leu-Glu-
Thr-Thr-Glu-Cys-Ile-Gln-Gly-Gln-Gly-Glu-Gly-Tyr-Arg-Gly-Thr-Val-Asn-Thr-
Ile-Trp-Asn-Gly-Ile-Pro-Cys-Gln-Arg-Trp-Asp-Ser-Gln-Tyr-Pro-His-Glu-His-
Asp-Met-Thr-Pro-Glu-Asn-Phe-Lys-Cys-Lys-Asp-Leu-Arg-Glu-Asn-Tyr-Cys-Arg-
Asn-Pro-Asp-Gly-Ser-Glu-Ser-Pro-Trp-Cys-Phe-Thr-Thr-Asp-Pro-Asn-Ile-Arg-
Val-Gly-Tyr-Cys-Ser-Gln-Ile-Pro-Asn-Cys-Asp-Met-Ser-His-Gly-Gln-Asp-Cys-
Tyr-Arg-Gly-Asn-Gly-Lys-Asn-Tyr-Met-Gly-Asn-Leu-Ser-Gln-Thr-Arg-Ser-Gly-
Leu-Thr-Cys-Ser-Met-Trp-Asp-Lys-Asn-Met-Glu-Asp-Leu-His-Arg-His-Ile-Phe-
Trp-Glu-Pro-Asp-Ala-Ser-Lys-Leu-Asn-Glu-Asn-Tyr-Cys-Arg-Asn-Pro-Asp-Asp-
Asp-Ala-His-Gly-Pro-Trp-Cys-Tyr-Thr-Gly-Asn-Pro-Leu-Ile-Pro-Trp-Asp-Tyr-
Cys-Pro-Ile-Ser-Arg-Cys-Glu-Gly-Asp-Thr-Thr-Pro-Thr-Ile-Val-Asn-Leu-Asp-
His-Pro-Val-Ile-Ser-Cys-Ala-Lys-Thr-Lys-Gln-Leu-Arg-Val-Val-Asn-Gly-Ile-
Pro-Thr-Arg-Thr-Asn-Ile-Gly-Trp-Met-Val-Ser-Leu-Arg-Tyr-Arg-Asn-Lys-His-
Ile-Cys-Gly-Gly-Ser-Leu-Ile-Lys-Glu-Ser-Trp-Val-Leu-Thr-Ala-Arg-Gln-Cys-
Phe-Pro-Ser-Arg-Asp-Leu-Lys-Asp-Tyr-Glu-Ala-Trp-Leu-Gly-Ile-His-Asp-Val-
His-Gly-Arg-Gly-Asp-Glu-Lys-Cys-Lys-Gln-Val-Leu-Asn-Val-Ser-Gln-Leu-Val-
Tyr-Gly-Pro-Glu-Gly-Ser-Asp-Leu-Val-Leu-Met-Lys-Leu-Ala-Arg-Pro-Ala-Val-
Leu-Asp-Asp-Phe-Val-Ser-Thr-Ile-Asp-Leu-Pro-Asn-Tyr-Gly-Cys-Thr-Ile-Pro-
Glu-Lys-Thr-Ser-Cys-Ser-Val-Tyr-Gly-Trp-Gly-Tyr-Thr-Gly-Leu-Ile-Asn-Tyr-
Asp-Gly-Leu-Leu-Arg-Val-Ala-His-Leu-Tyr-Ile-Met-Gly-Asn-Glu-Lys-Cys-Ser-
Gln-His-His-Arg-Gly-Lys-Val-Thr-Leu-Asn-Glu-Ser-Glu-Ile-Cys-Ala-Gly-Ala-
Glu-Lys-Ile-Gly-Ser-Gly-Pro-Cys-Glu-Gly-Asp-Tyr-Gly-Gly-Pro-Leu-Val-Cys-
Glu-Gln-His-Lys-Met-Arg-Met-Val-Leu-Gly-Val-Ile-Val-Pro-Gly-Arg-Gly-Cys-
Ala-Ile-Pro-Asn-Arg-Pro-Gly-Ile-Phe-Val-Arg-Val-Ala-Tyr-Tyr-Ala-Lys-Trp-
Ile-His-Lys-Ile-Ile-Leu-Thr-Tyr-Lys-Val-Pro-Gln-Ser

Figure 23

Pep21

Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp

Pep22

$HOOCCH_2CH_2CONH$-Asp-Glu-Glu-Ala-Val-Tyr-Phe-Ala-His-Leu-Asp-Ile-Ile-Trp

Pep23

Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr

Pep24

Gly-Leu-Phe-Glu-Ala-Ile-Ala-Asp-Phe-Ile-Glu-Asn-Gly-Trp-Glu-Gly-Met-Ile-Asp-Gly-Gly-Gly-Cys

Pep25

Lys-Val-Tyr-Thr-Gly-Val-Tyr-Pro-Phe-Met-Trp-Gly-Gly-Ala-Tyr-Cys-Phe-Cys-Asp

Pep26

Gly-Gly-Tyr-Cys-Leu-Thr-Arg-Trp-Met-Leu-Ile-Glu-Ala-Glu-Leu-Lys-Cys-Phe-Gly-Asn-Thr-Ala-Val

Figure 23

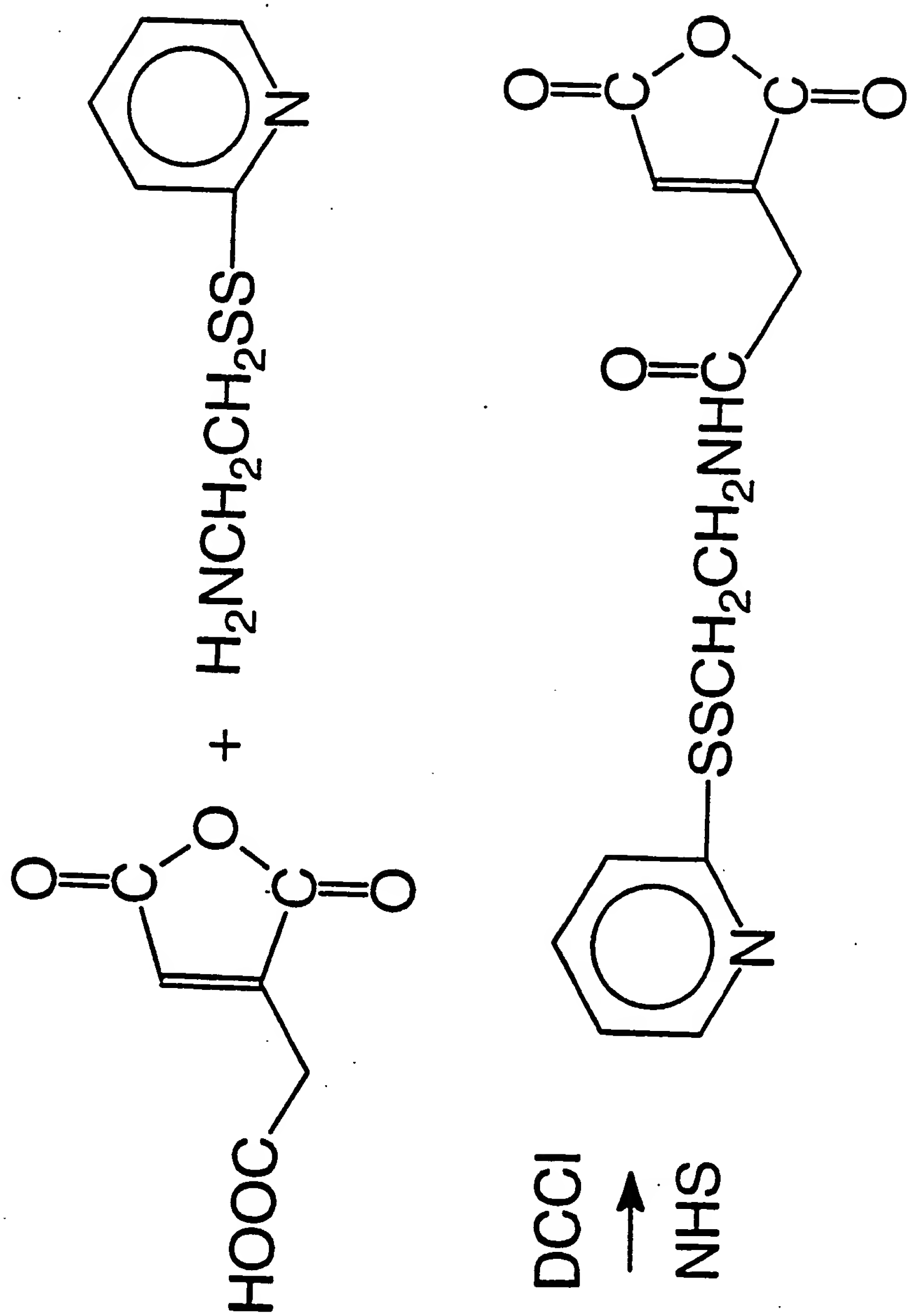


Figure 24

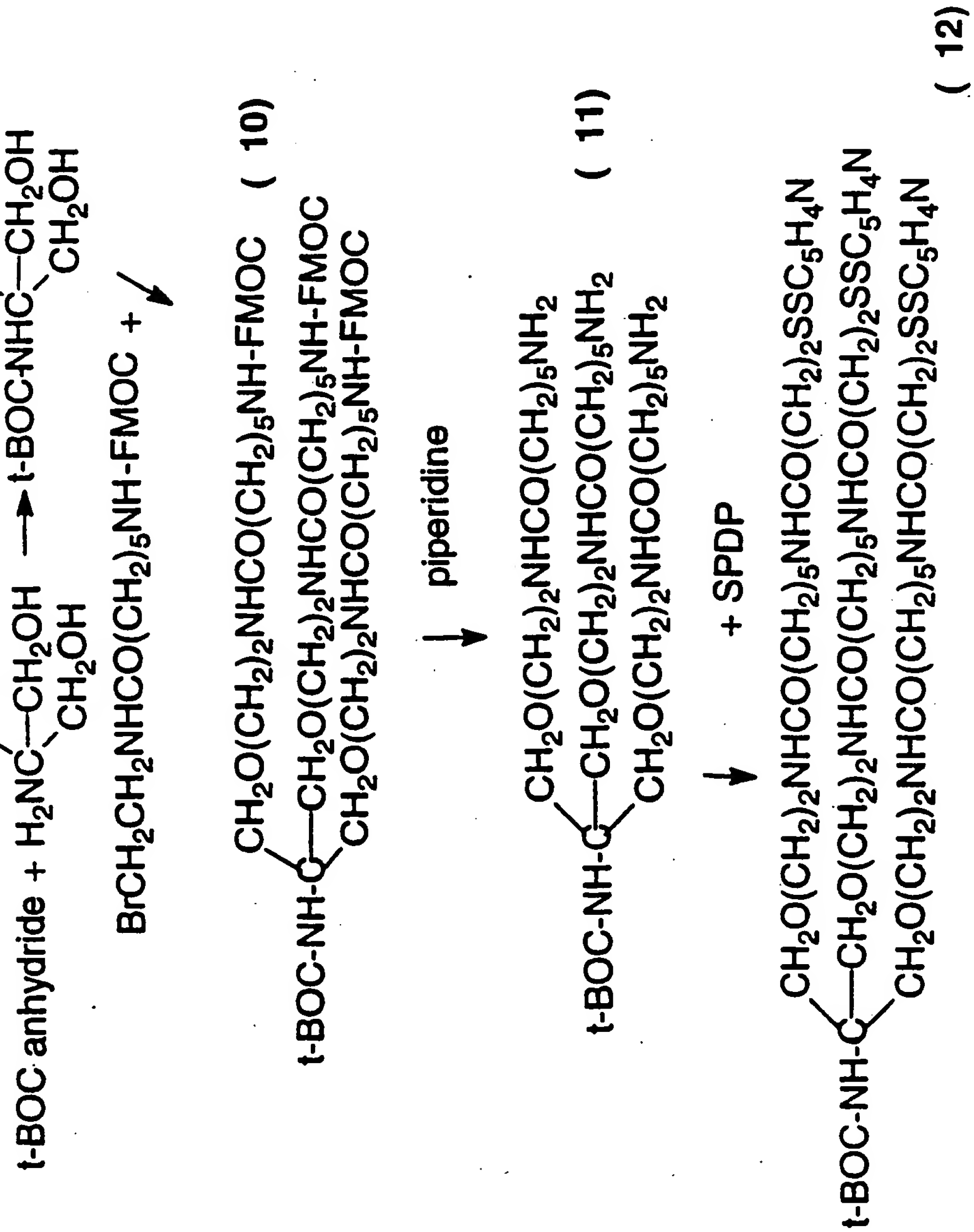
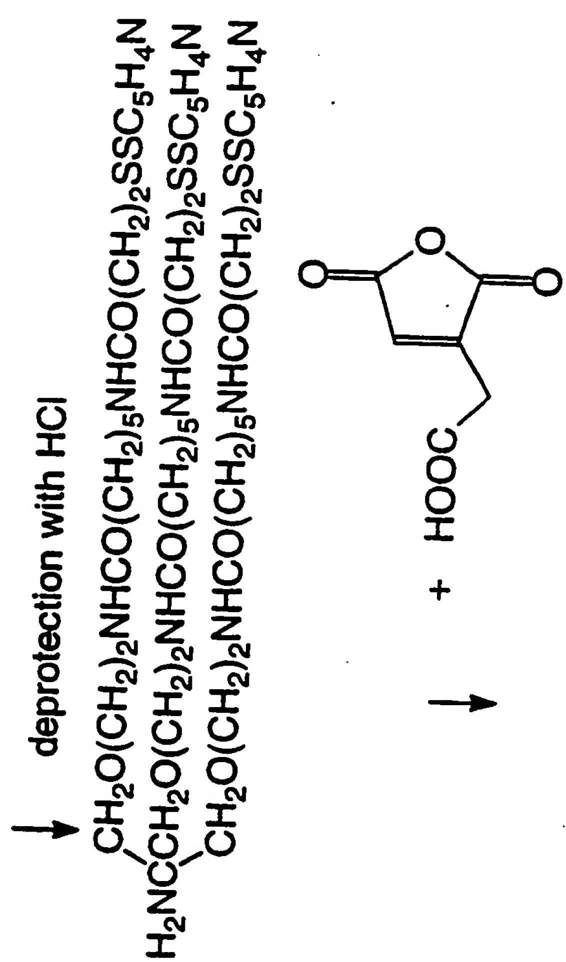


Figure 25



(13)

Figure 25

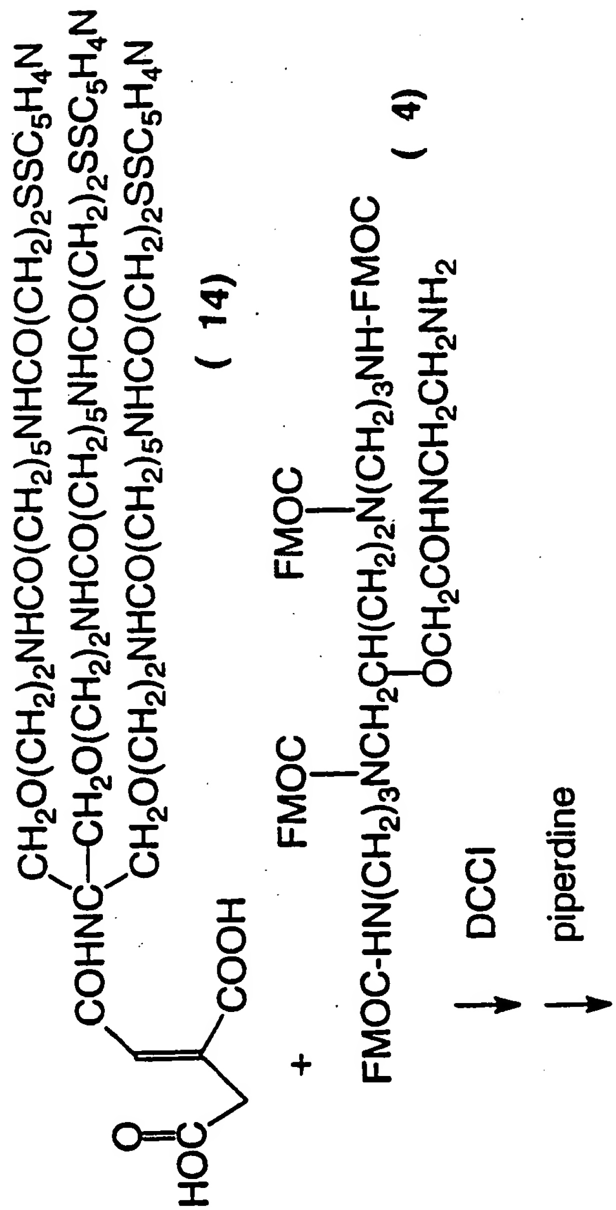

CH2O(CH2)2NHCO(CH2)5NHCO(CH2)2SSC5H4N
COHNC—CH2O(CH2)2NHCO(CH2)5NHCO(CH2)2SSC5H4N
CH2O(CH2)2NHCO(CH2)5NHCO(CH2)2SSC5H4N
HOC
O
COOH
(14)
+
FMOC
FMOC
FMOC-HN(CH2)3NCH2CH(CH2)2N(CH2)3NH-FMOC
OCH2COHNCH2CH2NH2
(4)
DCCI
piperdine

Figure 25

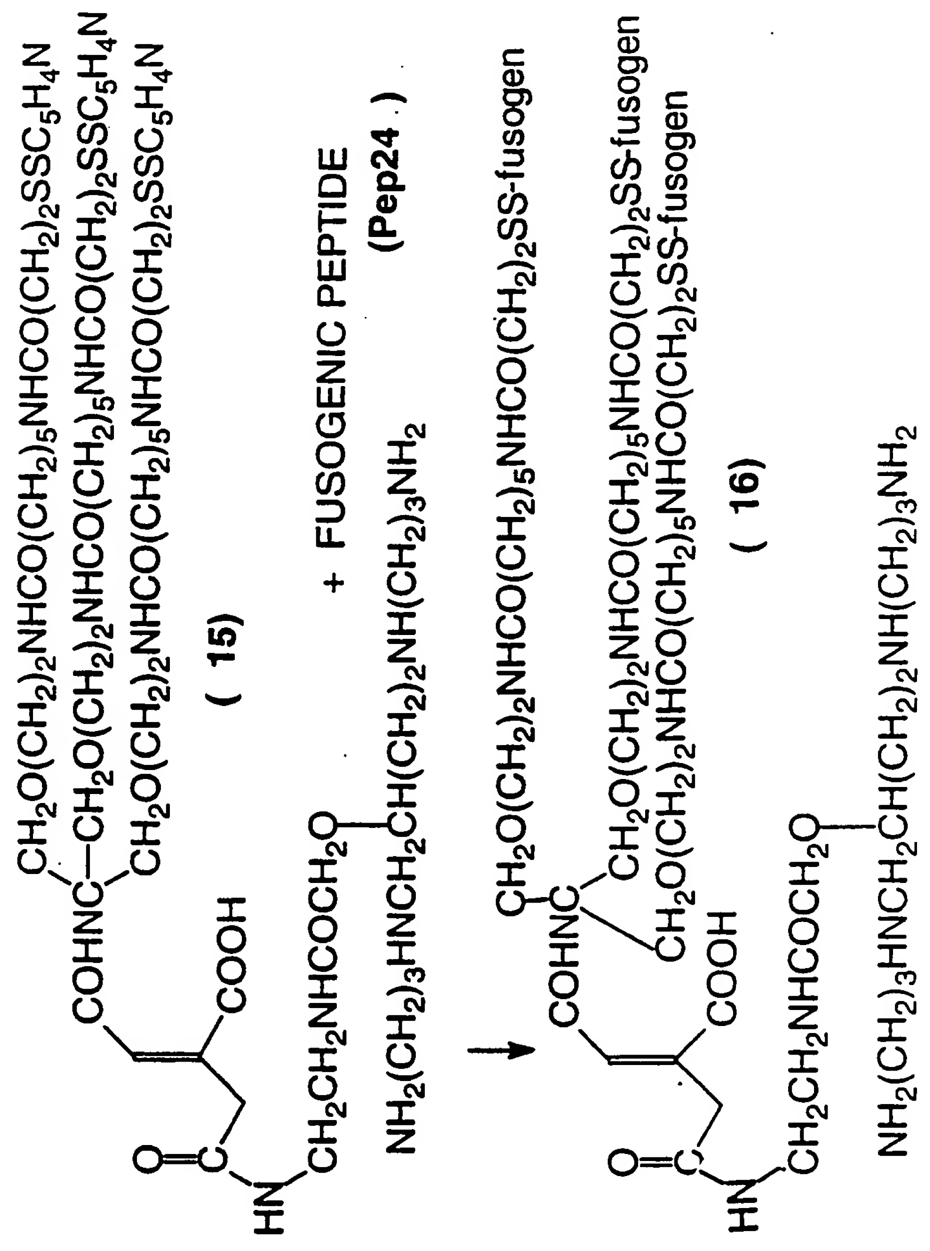
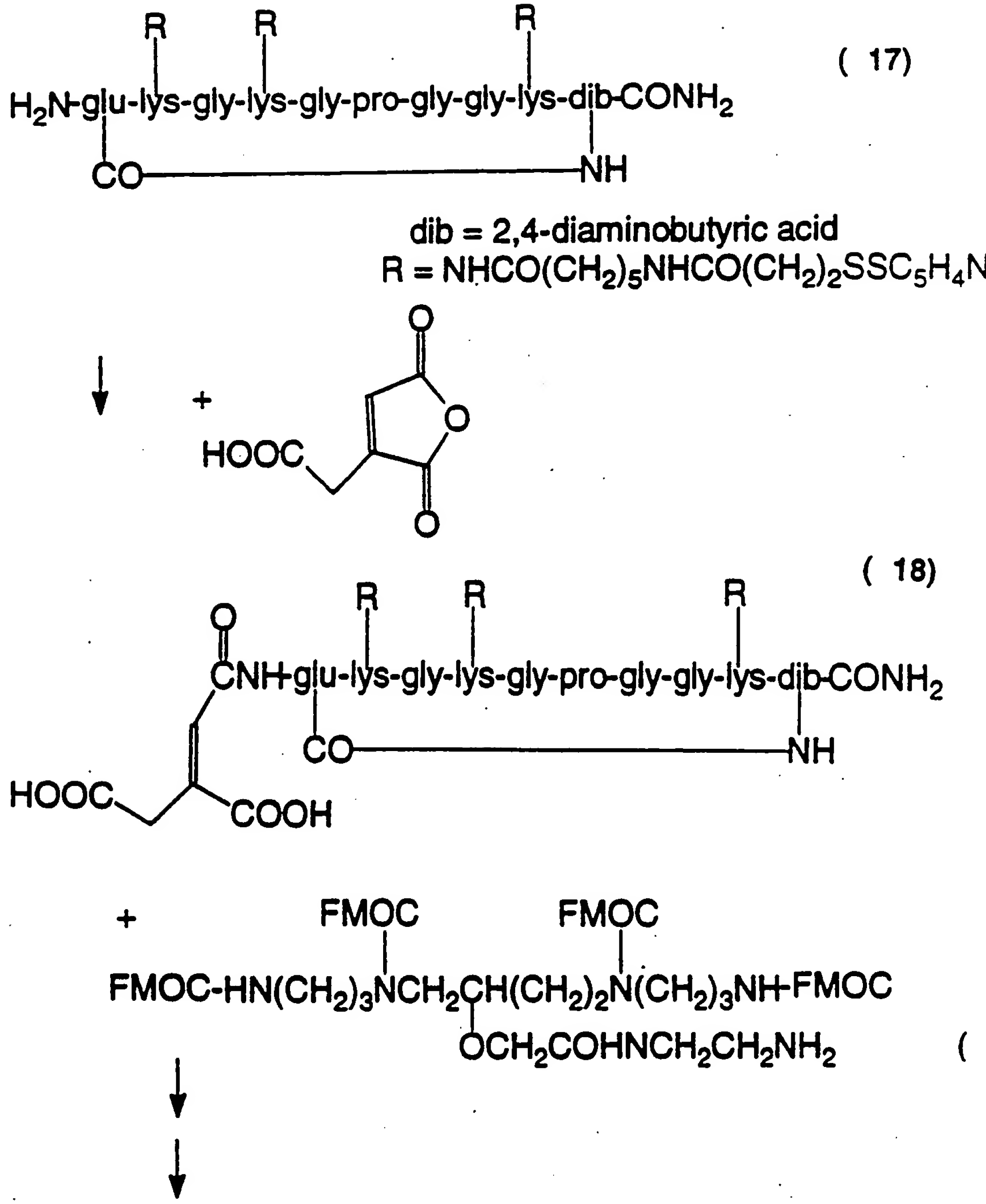


Figure 25



(17)

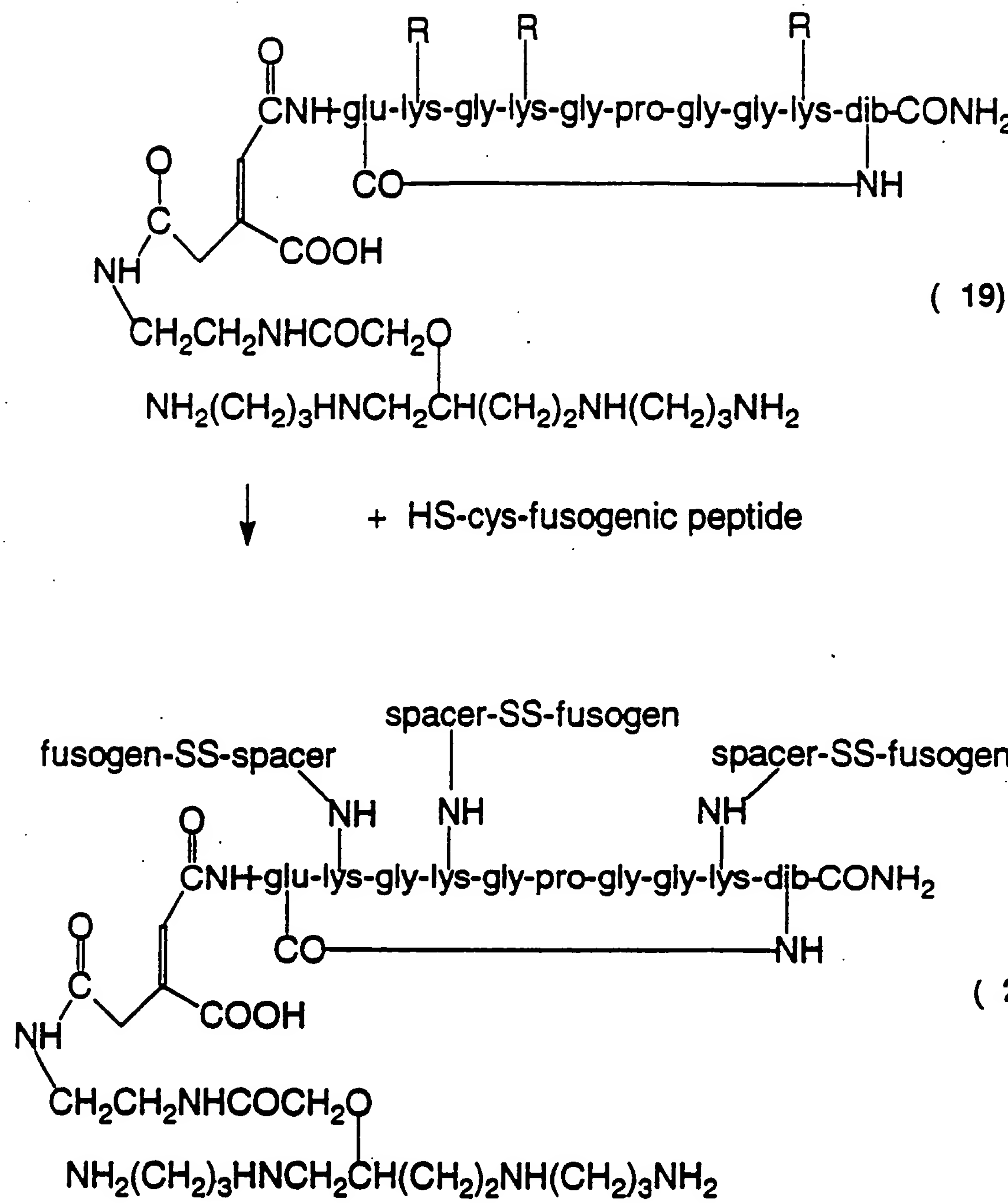
dib = 2,4-diaminobutyric acid

R = $NHCO(CH_2)_5NHCO(CH_2)_2SSC_5H_4N$

(18)

(4)

Figure 26



(19)

(20)

where dib = 2,4-diaminobutyric acid

Figure 26

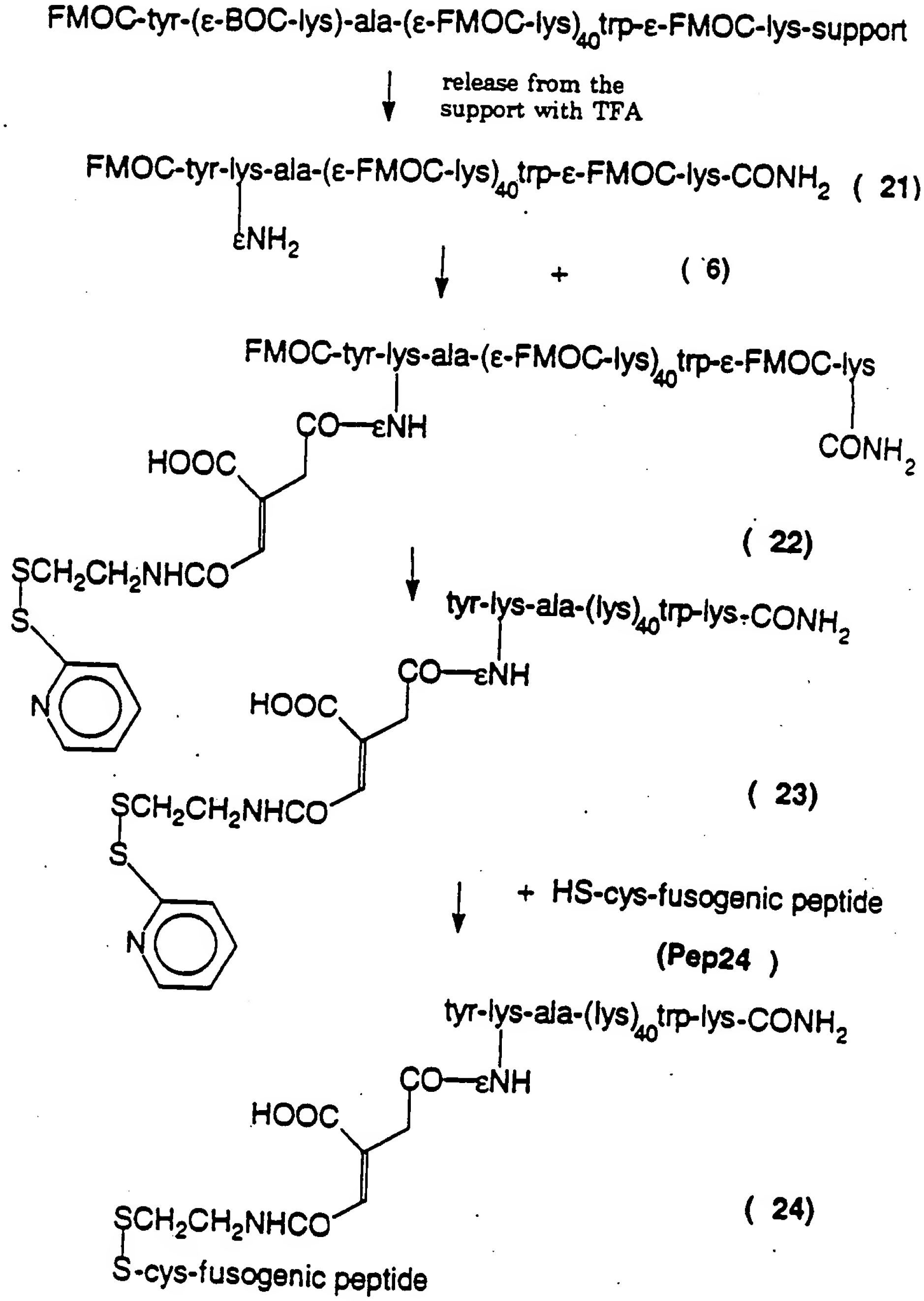
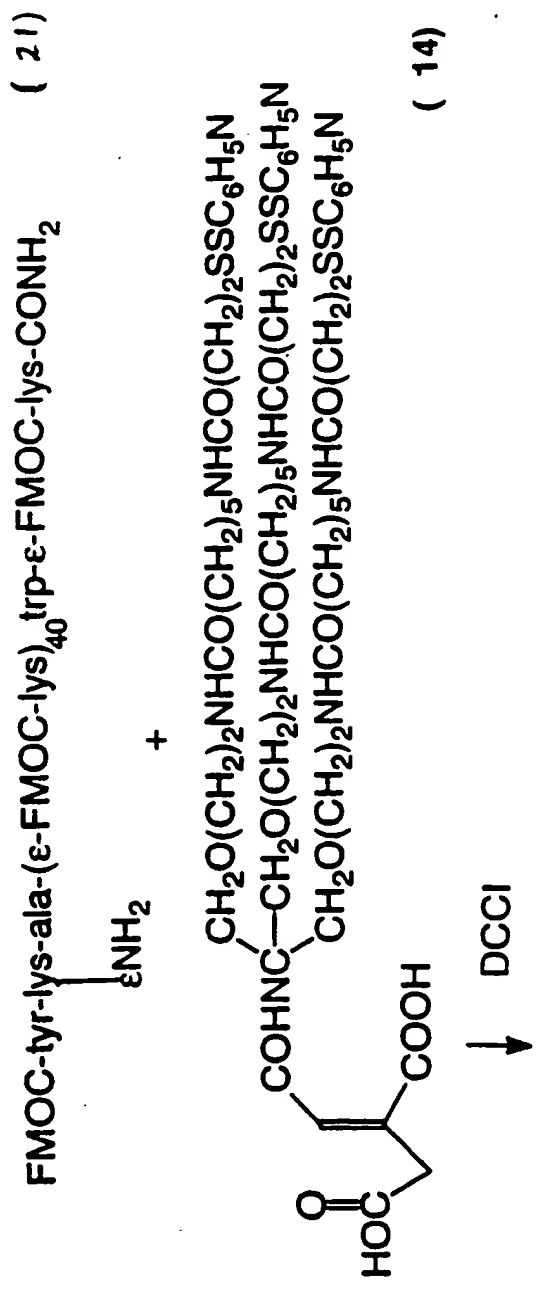
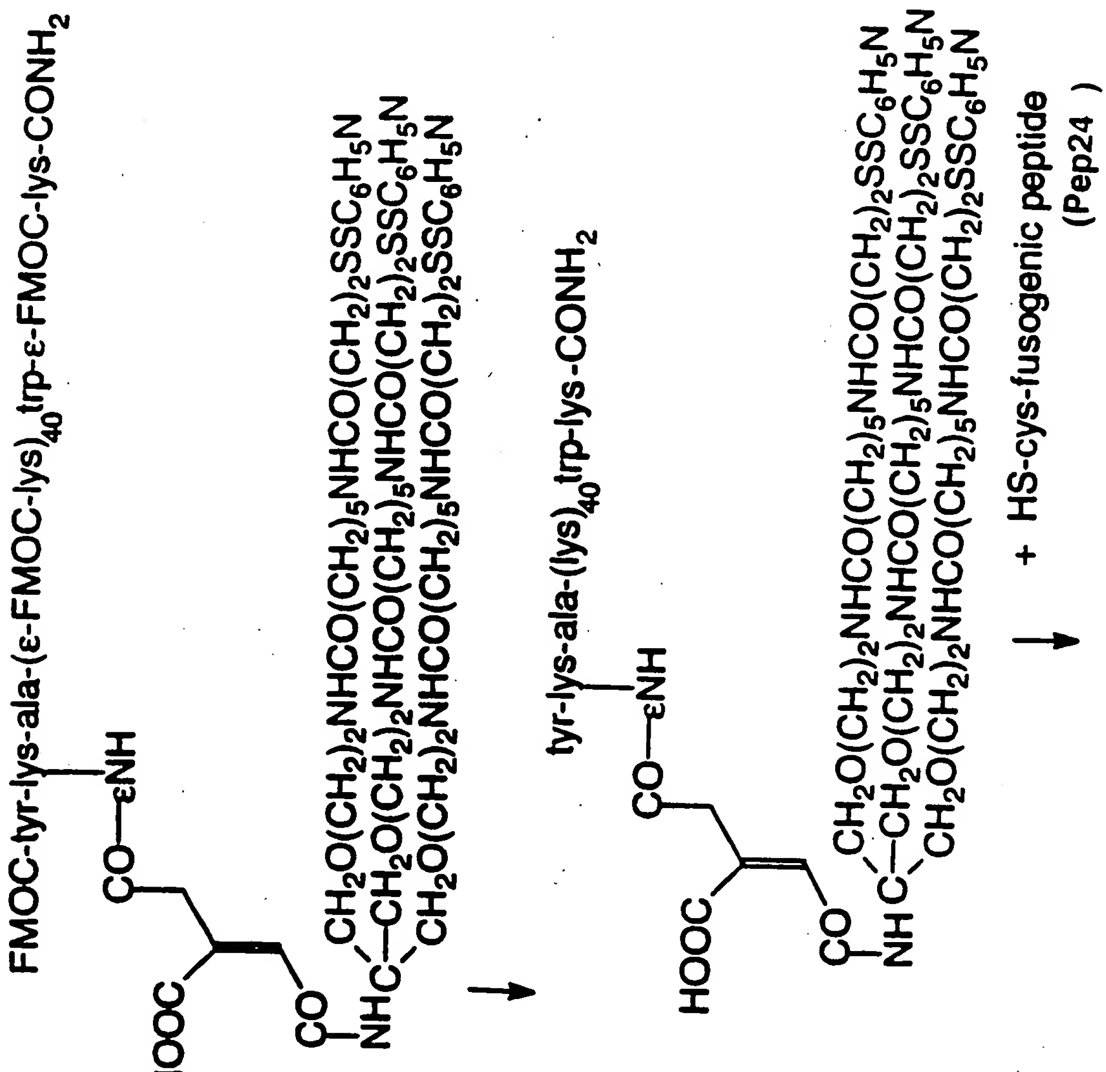

FMOC-tyr-(ε-BOC-lys)-ala-(ε-FMOC-lys)40trp-ε-FMOC-lys-support
release from the support with TFA
FMOC-tyr-lys-ala-(ε-FMOC-lys)40trp-ε-FMOC-lys-CONH2 (21)
εNH2
+ (6)
FMOC-tyr-lys-ala-(ε-FMOC-lys)40trp-ε-FMOC-lys
CO—εNH
HOOC
CONH2
SCH2CH2NHCO
S
N
(22)
tyr-lys-ala-(lys)40trp-lys-CONH2
CO—εNH
HOOC
SCH2CH2NHCO
S
N
(23)
+ HS-cys-fusogenic peptide
(Pep24)
tyr-lys-ala-(lys)40trp-lys-CONH2
CO—εNH
HOOC
SCH2CH2NHCO
S-cys-fusogenic peptide
(24)

Figure 27



Figu' '9

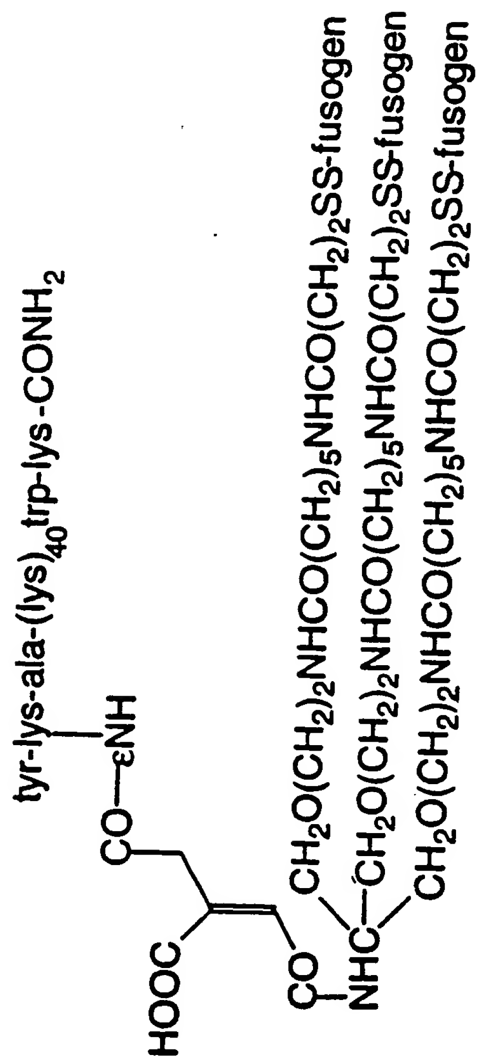


(25)

(26)

Figure 28

(27)



Figu

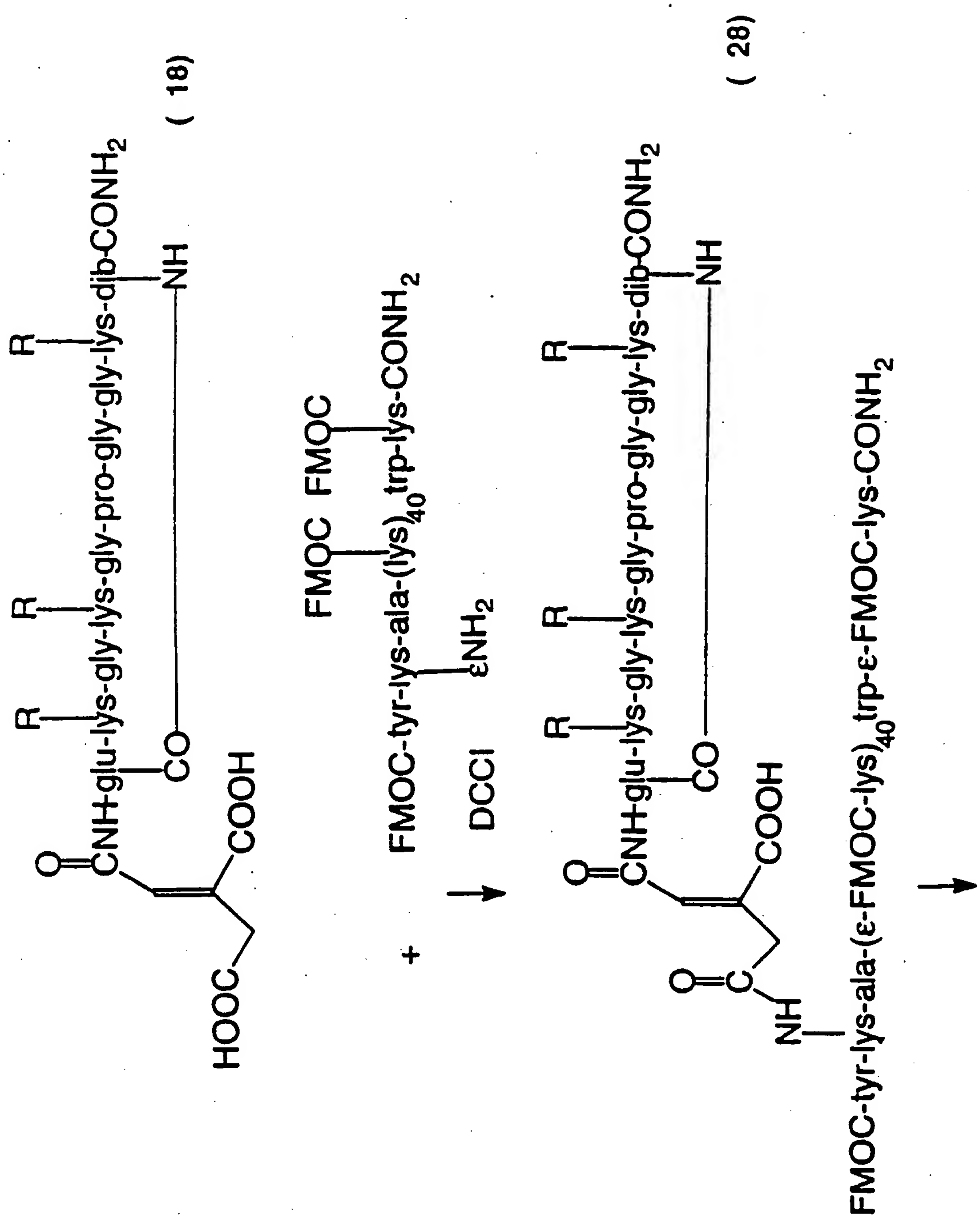


Figure 29

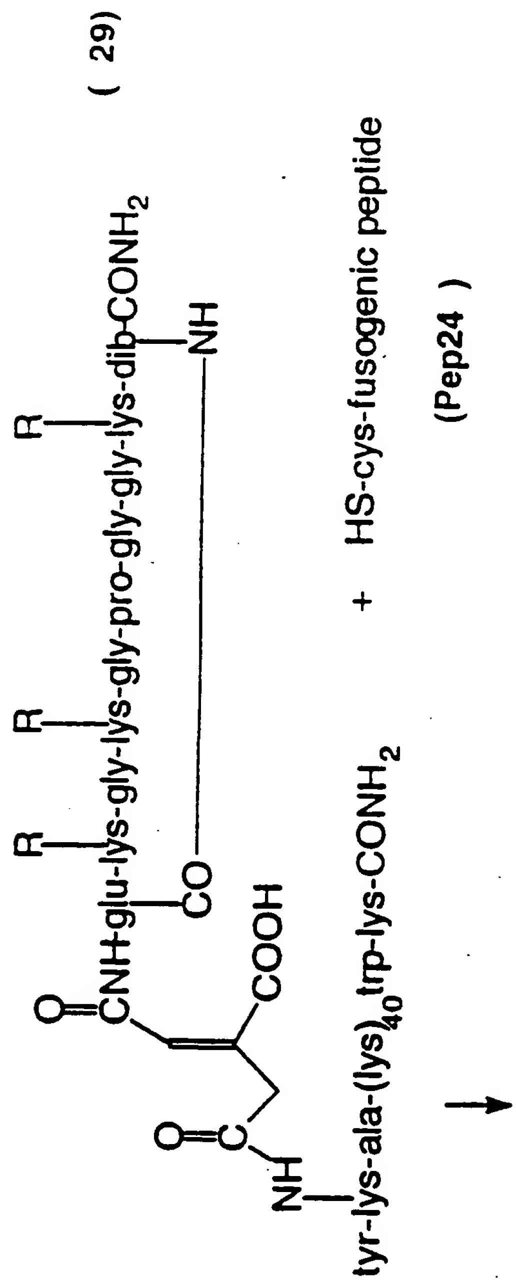
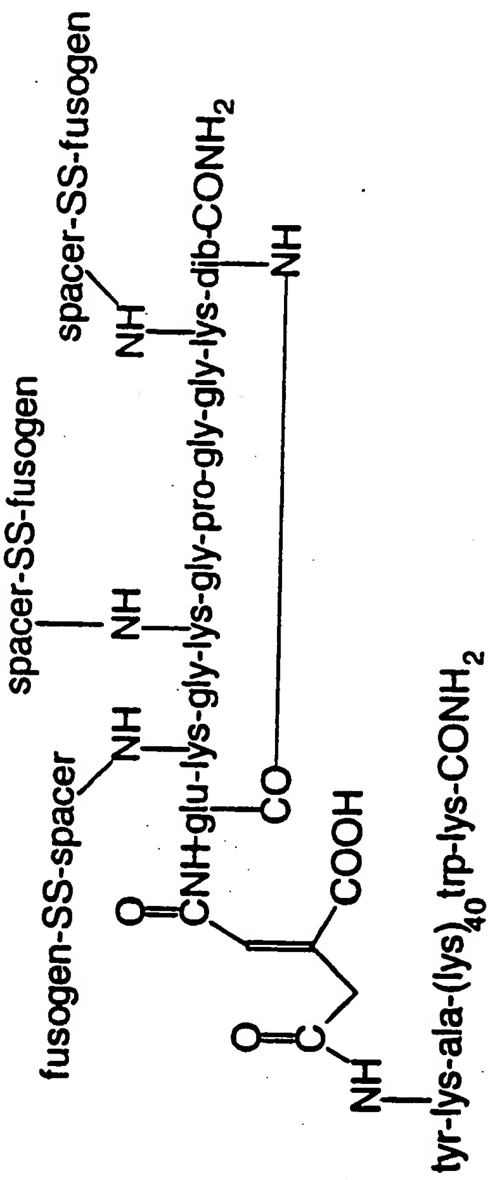

CNH-glu-lys-gly-lys-gly-pro-gly-gly-lys-dib-CONH2
R
R
R
CO
NH
COOH
NH
C
O
O
tyr-lys-ala-(lys)40 trp-lys-CONH2
(29)
+ HS-cys-fusogenic peptide
(Pep24)

Figure 29

(30)



where dib = 2,4-diaminobutyric acid

Figure 29

INTERNATIC . SEARCH REPORT

International application No. .CT/US93/02725

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :424/85.8, 93R, 93B, 94.1; 435/172.3, 514/2, 4, 17, 18, 19, 44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,801,575 (PARDRIDGE) 01 JANUARY 1989, see entire document.	1-111
Y	US, A, 4,891,219 (KARR et al.) 02 JANUARY 1990, see entire document.	1-111
Y,P	US, A, 5,108,921 (LOW et al.) 28 APRIL 1992, see entire document.	1-111
Y,P	US, A, 5,166,320 (WU et al.) 24 NOVEMBER 1992, see entire document.	1-111

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 MAY 1993	16 JUN 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer BRIAN R. STANTON
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCONJUGATE CHEMISTRY, Volume 2, No.4, issued 1991, E. Wagner et al., "DNA-Binding Transferrin Conjugates as Functional Gene-Delivery Agents: Synthesis of Linkage of Polylysine or Ethidium Homodimer to the Transferrin Carbohydrate Moiety", pages 226-231, see entire document.	1-111
Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 88, issued October 1991, D.T. Curiel et al., "Adenovirus enhancement of transferrin-polylysine-mediated gene delivery", pages 8850-8854, see entire document.	1-111
Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 88, issued May 1991, E. Wagner et al., "Transferrin-polycation-DNA complexes: The effect of polycations on the structure of the complex and DNA delivery to cells", pages 4255-4259, see entire document, especially pages 4255 and 4256, <u>Materials and Methods</u>.	1-111
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 262, Number 10, issued 05 April 1987, G.Y. Wu et al., "Receptor-mediated *in Vitro* gene Transformation by a Soluble DNA Carrier System", pages 4429-4432, see entire document, especially pages <u>Abstract</u> and <u>Experimental Procedures</u>.	1-111
Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 86, issued September 1989, L.A. Yakubov et al., "Mechanism of oligonucleotide uptake by cells: Involvement of specific receptors?", pages 6454-6458, see entire document, especially <u>Materials and Methods</u>.	1-111
Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 87, issued June 1990, M. Cotten et al., "Transferrin-polycation-mediated introduction of DNA into human leukemic cells: Stimulation by agents that affect the survival of transfected DNA or modulate transferrin receptor levels", pages 4033-4037, see entire document, especially <u>Abstract</u> and <u>Materials and methods</u>.	1-111
Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCE, USA, Volume 87, issued May 1990, E. Wagner et al., "Transferrin-polycation conjugates as carriers for DNA uptake into cells", pages 3410-3414, see entire document, especially <u>Materials and Methods</u> and Figure 1.	1-111

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTICANCER RESEARCH, volume 11, issued 1991, K. Shitara et al., "Application of Anti-Sialyl Le[a] Monoclonal antibody, KM231, for Immunotherapy of Cancer", pages 2003-2014, see entire article, especially Materials and Methods.	1-111
Y	CELL REGULATION, volume 1, issued March 1990, T.E. McGraw et al., "Human transferrin receptor internalization is partially dependent upon an aromatic amino acid on the cytoplasmic domain", pages 369-377, see entire article, especially Figures 1-4 and Tables 1-3.	1-111
Y	BIOTHERAPY, volume 3, issued 1991, G.Y. Wu et al., "Delivery systems for gene therapy", pages 87-95, see entire document.	1-111
Y	AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY, volume 6, issued 1992, D.T. Curiel et al., "Gene Transfer to Respiratory Epithelial Cells via the Receptor-mediated Endocytosis Pathway", pages 247-252, see entire document, especially Abstract and Materials and Methods.	1-111
Y	NATURE, volume 349, issued 24 January 1991, P.L. Felgner et al., "Gene therapeutics", pages 351-352, see entire article, especially Table 1.	43-48
Y	SCIENCE, volume 244, issued 16 June 1989, T. Friedman, "Progress Toward Human Gene Therapy", pages 1275-1281, see especially section entitled Direct Vector Delivery in Vivo.	43-48
Y	BIOCONJUGATE CHEMISTRY, Volume 1, issued 1990, J-P, Leonetti et al., "Biological Activity of Oligonucleotide-Poly(L-lysine) Conjugates: Mechanism of Cell Uptake", pages 149-153, see entire document especially Experimental Procedures.	1-111

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02725

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 31/01, 37/02, 37/48, 47/02, 47/06, 47/08, 47/10, 47/12, 48/00, 49/00; C12N 5/00, 5/6, 5/8, 5/10, 7/04, 15/00, 15/06, 15/07, 15/11, 15/87, 15/88, 15/89, 15/90

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS, STN-Medline, Biosis, Registry, Genebank
Search Terms:Spermin?, DNA, Binding, Polyamine?, Poly, Amine?, Hydroxyspermine?, Hydroxy (w) Spermine?, Polylysine?, Asialoglycoprotein, Transferrin, Gene, Transfection, Transformation, Delivery, Transfer, Introduction, Peptide structure search in STN-registry

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-38, 94-98 and 43, drawn to multi-component therapeutic compositions and methods of use, classified in Class 514, various subclasses and Class 435, Subclass 172.3.

II. Claims 39-42, drawn to single component transporter systems, classified in Class 514, various subclasses.

III. Claims 44-48, drawn to methods of *in vivo* gene therapy, classified in Class 514 subclass 44.

IV. Claims 49-93 and 99-111, drawn to compounds, classified in Class 530, subclass 300.

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Telephone Practice)
Please See Extra Sheet.

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.